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Studies on the Cuticle of the Grasshopper *Melanoplus bivittatus* (Say) (Orthoptera: Acrididae)

I. General Properties of the Cuticle[1]

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Introduction

Numerous studies have shown that the cuticle of insects is complex and highly-differentiated and that the complexity varies from species to species (2, 8, 11, 13, 14, 17, 18).

The object of this study is to determine cuticular relationships between the grasshopper, *Melanoplus bivittatus*, and other insects already studied. This work was carried out by means of chemical tests and permeability measurements on fresh material rather than by methods of fixing and sectioning.

Methods and Materials

The permeability of the cuticle in this study was measured according to Wigglesworth (16). The grasshoppers were killed in hydrogen cyanide to which were added a few drops of ammonium hydroxide. This ensured rapid death and prevented the loss of body fluids by regurgitation. The dead grasshoppers were placed in a small metal gauze basket which was suspended in a conical flask over phosphorus pentoxide. The bulb of a thermometer, which recorded the temperature within this flask, was near the basket containing the grasshoppers. The effects of a humidity gradient in the immediate vicinity of the insects were minimized by agitation of the air in the chamber by means of a mechanical glass stirrer propelled by compressed air. Although the speed of this stirrer was not controlled precisely, it was possible, after some experience, to maintain fairly constant motion during the course of the experiment.

The difference in weight of the grasshoppers before and after exposure to the dry air was assumed to be due to the loss of moisture by evaporation, predominantly through the cuticle (4, 10).

Only grasshoppers which had passed their third day after moulting were used. This was done to avoid, as much as possible, complications which might arise from continued secretion and tanning of the endocuticle during the first few days after ecdysis.

Results

(a) *The lipid epicuticle*

When dead grasshoppers are immersed in a half-percent aqueous solution of osmic acid or exposed to its fumes, the cuticle turns black. A similar treatment with a methylal solution of Sudan III resulted in a reddish cuticle. Because both reactions are characteristic of lipids, it appears that the surface of the grasshopper cuticle is covered by a lipid coating, which will be called the lipid epicuticle (11).

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TABLE I
Evaporation from adult grasshopper exposed to dry air at 40° C. for 15 minutes, after various treatments

Treatment	Per Cent Loss of Weight
Untreated	0.76
After abrasion with fine emery	1.7
Extracted with cold chloroform, 10 minutes	6.0

If this is true, then wax solvents such as chloroform or detergents, or abrasion should remove this coating. The results presented in Table I show that treatment of the cuticle with chloroform or by abrasion resulted in increased permeability of the exoskeleton. As a result of partial abrasion of the abdomen, there occurred a two-fold increase in water permeation; treatment with cold chloroform gave approximately an eight-fold increase. This increased transpiration demonstrates that the lipid epicuticle is the protective cuticular component against desiccation.

The data in Table II show that untreated cuticles gave negative argentaffin and xanthoproteic tests. Only the tibial spurs reacted positively. When the lipid epicuticle was removed, the cuticle reacted positively to both tests, suggesting that beneath the lipid epicuticle are substances containing reducing functional groups as well as substances containing a benzene nucleus. The latter could be tyrosine and tryptophane either free or as residues of cuticular proteins.

The lipid epicuticle was not removed completely after treatment with the detergent. It was however, removed completely by chloroform, indicating perhaps that in certain regions of the cuticle the detergent was unable to break the bonds between the wax and its substrate. It appears improbable that the detergent affected the cement layer for, as will be shown later, no evidence for the existence of such a layer in this insect could be found. Wigglesworth (16) found that lipids from different species and even from different cuticular regions of the same species varied in their resistance to extraction with chloroform.

The above evidence is interpreted in support of the conclusion that the epicuticle of *Melanoplus bivittatus* consists of reducing substances which are covered by a protective coating of lipids.

(b) *Time of Deposition of the Lipid Epicuticle*

Evidence has been presented that the lipid epicuticle is mainly responsible for the impermeability of the cuticle of *Melanoplus bivittatus*. It has been found

TABLE II
The reaction of the cuticle to the argentaffin and xanthoproteic tests after various treatments

Treatment	Argentaffin test	Xanthoproteic test
Untreated	−	−
Abraded with emery paper	+	+
Chloroform — 10 minutes	+	+
"Lorol" sulfate detergent (25% solution)	+	

in *Rhodnius* and several species of ticks that the impermeability of the cuticle is well established before the old exoskeleton is shed (8, 17). In *Tenebrio* the lipid is completely secreted one day after moulting (18). The following experiments were performed to determine when in the moulting cycle of this grasshopper the lipid epicuticle is secreted.

Two lots of grasshoppers, one consisting of those which were dissected out of the old cuticle immediately before moulting and another consisting of those which had emerged from the nymphal exuvium for approximately twenty-four hours were exposed to dry air for twenty minutes at 25°C. before and after treatment with chloroform.

TABLE III

Loss of weight of fourth instar grasshopper nymphs exposed to dry air at 25°C. for 20 minutes

Treatment	Age	Per cent loss of weight
Control	Moulting	0.46
Cold chloroform	Moulting	33.3
Control	24 hrs. after moulting	0.37
Cold chloroform	24 hrs. after moulting	33.5

The data presented in Table III show that the permeability of the new cuticle within the nymphal exuvium is of the same order of magnitude as that of a cuticle 24 hours after emergence from the nymphal exuvium. The increase in the rate of permeation through the newly-formed cuticle after chloroform treatment indicates the presence of protective surface lipids. Untreated cuticles of either age, gave negative argentaffin and xanthoproteic tests.

Further evidence for the presence of lipid on the new cuticle within the nymphal skin was obtained from the similarity in values of contact angles of distilled water on the new and old cuticle. As a result of 25 measurements, the average contact angle on a new cuticle was found to be 105.9°. This compared favourably with an average of 105.0° for cuticles which were several days old. The contact angle did not appear to vary with location on the cuticle. By contrast, the average contact angle was 78° on a cuticle after it was treated with chloroform. It is therefore evident that in the absence of surface lipids the cuticle is hydrophilic; it becomes highly hydrophobic in the presence of a discrete layer of lipids.

TABLE IV

Loss of body weight of freshly-moulted adult grasshoppers exposed to dry air 30°C. for 20 minutes after being treated with chloroform at 25°C. for varying periods of time

Length of Treatment	Per Cent Loss of Body Weight
Controls	2.3
15-minute treatment	31.3
1-hour treatment	29.3
2-hour treatment	29.6

TABLE V
Per cent loss of moisture from treated grasshoppers held in dry air at 30°C. for one hour

Treatment	Treatment of Treatment, °C.	Per Cent Loss of Weight
Controls	25.0	2 0
Chloroform vapors	25 0	2 8
Chloroform	25.0	11 1
Chloroform	40.0	11.8

The evidence presented above is taken to support the conclusion that the lipid epicuticle of *M. bivittatus* is secreted before moulting is completed.

(c) *Determination of the Absence of a Cement Layer*

It has been shown by several investigators that the lipid epicuticle of some insects and ticks is protected by an outer cement layer (8, 13, 16, 17, 18). This cement layer is apparently tanned protein resistant to extraction by cold chloroform. It may, however, be disrupted by hot chloroform, thereby greatly increasing the permeability of the cuticle.

One of the factors which may be involved in the solution of the cement layer in cold chloroform is the duration of extraction. Three lots of newly-moulted grasshoppers were treated as indicated in Table IV, while the fourth lot was used as a control. Table IV shows that the duration of the extraction had no effect on the permeability of the cuticle. Thus, the loss of moisture from a grasshopper treated for two hours with cold chloroform was similar to the one treated for fifteen minutes. These results indicate that either the lipid epicuticle is covered by a very resistant protective coating or that the lipid epicuticle was removed completely by the fifteen minute treatment.

If the lipid epicuticle was protected by a coating resistant to extraction by cold chloroform, then it should be disrupted, at least in part, by extraction in hot chloroform as shown by Wigglesworth (16). Twenty grasshoppers, which were at least three days old were divided into four batches. One was used as a control and the others were treated as shown in Table V. After each treatment which lasted for thirty minutes, the insects were exposed to a dry atmosphere for one hour at 30°C.

Because the transition point of the wax of this grasshopper occurred at approximately 43°C. it was necessary to treat the insects with chloroform at 40°C. Otherwise any effects which would be obtained at any higher temperature could be attributed to the breakdown of the wax structure due to temperature and not to its extraction by the hot chloroform.

The results shown in Table V indicate that the permeability of the cuticle was increased approximately five-fold as a result of treatment with chloroform at 25 to 40°C. Chloroform vapors did not disturb the lipids of the grasshopper epicuticle to any extent. Hence if the lipids are to be removed, they must be in contact with chloroform. Treatment with hot chloroform did not remove any more wax than was removed by cold chloroform. Presumably all the wax capable of extraction was removed by chloroform at room temperature. Hence,

according to one of the criteria of Wigglesworth (16) it appears that the lipid epicuticle of this insect is not protected by any overlying component.

(d) *The Lipoprotein Epicuticle*

It has been found that in the cuticle of insects there are chemically bound lipids as well as a discrete lipid fraction (5, 11, 15, 17). In order to test for the presence of bound lipids in the grasshopper epicuticle, ten exuviae from fifth instar *M. bivittatus* were divided into five lots of two each. The first lot, used as a control, was exposed to fumes of a one-half per cent aqueous solution of osmic acid. After approximately an hour the skins assumed a black color, indicating the presence of a fatty material. The other four lots were extracted with chloroform at 53°C. for two hours, seven hours, three days, and eight days, respectively. After each period of extraction the chloroform was allowed to evaporate and the skins were exposed to fumes of one-half per cent osmic acid solution. In every instance the skins stained black, indicating the presence of unremovable lipids. Hurst (5) has found the presence of thermo-stabile bound lipids in the *Calliphora* epicuticle. Because saturated lipids do not give a positive test with osmic acid (12) it appears that at least some of the bound lipids of the grasshopped epicuticle are unsaturated. Furthermore, cast skins also gave a positive xanthoproteic test, indicating the presence of proteins.

If nymphal skins are treated with cold chloroform and are then warmed very gently with nitric acid and potassium chlorate there occurs at first a vigorous evolution of gas. Further warming results in a disintegration of the membrane with the release of apparently oily droplets which are themselves very readily oxidized.

These results suggest that the exuviae contain unextractable lipids which are chemically bound to proteins. This lipoprotein fraction of the epicuticle is probably analogous to the cuticulin layer of *Rhodnius* and other insects (17, 18).

Discussion

The present study indicates that the epicuticle of the grasshopper *Melanoplus bivittatus* consists of two fractions: a discrete lipid epicuticle which is readily removed by either hot or cold chloroform but not chloroform vapours and a lipoprotein epicuticle composed of lipids bound to proteins. The bound lipids cannot be removed even after exhaustive extraction by hot chloroform.

It is well known that many substances such as melanin, aldehydes, adrenalin (3), polyphenols, polyamines, aminophenols, proteins (9), unsaturated lipids (1), urates, and purine derivatives (6) give a positive reaction with ammoniacal silver nitrate. Although such a positive reaction was obtained in this study under certain conditions, and has been used by some workers as evidence for the presence of polyphenols and a polyphenol layer (8, 13, 16, 17, 18), it is felt that, in view of the unspecificity of this reaction, the assumption that it is indicative of polyphenols requires a more critical study. It may be that this reaction simply indicates the presence of reducing compounds and/or functional groups which may or may not have a laminar orientation within the cuticle.

This study indicates that, as for many terrestrial insects so for the grasshopper, the most essential feature in its water economy is the lipid epicuticle. Damage to or removal of this fraction of the epicuticle in the grasshopper resulted in a rapid loss of body moisture, leading ultimately to desiccation. It is therefore the discrete lipid component that confers most of the impermeability on the grasshopper cuticle. The lipids which are chemically bound to the proteins of

the cuticle do cause a suppression in evaporation when compared to that of a free surface. This will be shown in the following communication. However, the rate of evaporation through a cuticle free of its lipid epicuticle, is still so great that the lipoprotein epicuticle probably plays a negligible role in water-proofing the cuticle of this grasshopper and probably many terrestrial insects.

Thus, although there is little doubt as to the component which is most important in the water economy of insects, yet the mechanism of this impermeability cannot be understood unless the orientation, thickness, degree of crystallization and bonding of the wax by the substrate are considered. It is particularly interesting to note that apparently the strength with which the proteinaceous substrate binds the wax is not the same throughout the cuticle. The detergent used in these experiments was capable of removing the wax more readily from certain regions of the grasshopper cuticle than from others. This may indicate strong regional bonding between the wax and its substrate and may be an important factor in determining the extent to which the wax will be removed by organic solvents.

In addition to the properties of the wax which may influence cuticular permeability, other properties of the cuticle such as its thickness, degree of tanning, and ultrastructure may also be important. An association has already been suggested between permeability and tanning (14). The importance and influence of cuticular ultrastructure on its permeability must await further research.

Acknowledgments

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Summary

By means of chemical tests and permeability measurements it was established that the epicuticle of *Melanoplus bivittatus* is composed of an outer lipid and an inner lipoprotein component. The lipid epicuticle is the main barrier to permeation of body moisture through the cuticle and is deposited on the new cuticle before ecdysis.

The "cement" layer, which in some insects presumably covers and protects the wax layer, is apparently absent in this grasshopper.

Although a positive argentaffin reaction was obtained with the epicuticle, it is felt that, because of the unspecificity of this reaction, it cannot be used in support of the occurrence of a polyphenol layer.

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II. Permeability of the Cuticle to Water at Various Temperatures[1]

Introduction

In a previous communication of this series (4), some general properties of the epicuticle of the two-striped grasshopper *Melanoplus bivittatus* were deduced from permeability measurements and chemical tests under various conditions. Among other things, it was found that the epicuticle is covered by a lipid layer, which regulates the rate of permeation of moisture through the cuticle. Because it has already been shown (2, 17, 21, 23, 24) that the rate of evaporation from the cuticle of several insects is a function of temperature and age, a study was undertaken of the effect of these factors upon the transpiration through the cuticle of grasshoppers in various instars.

The work of others (2, 17, 24) has been extended by the finding that the rate of evaporation-temperature curves are characterized by "breaks" not limited to a particular temperature, referred to as the critical temperature, but to a temperature interval which will be termed the "transition region" of the wax. Furthemore, Arrhenius plots of these curves suggest that the permeability of such complex biological membranes probably involves several factors such as diffusion and solution of the penetrating substance within the membrane. Although the data on this point are not complete, it is felt that the results indicate an approach which may be helpful in future studies of membrane permeability.

Methods and Materials

The grasshoppers used in this study were reared at 29.5-0.3°C. from field-collected eggs, according to the method described previously (4). Grasshoppers

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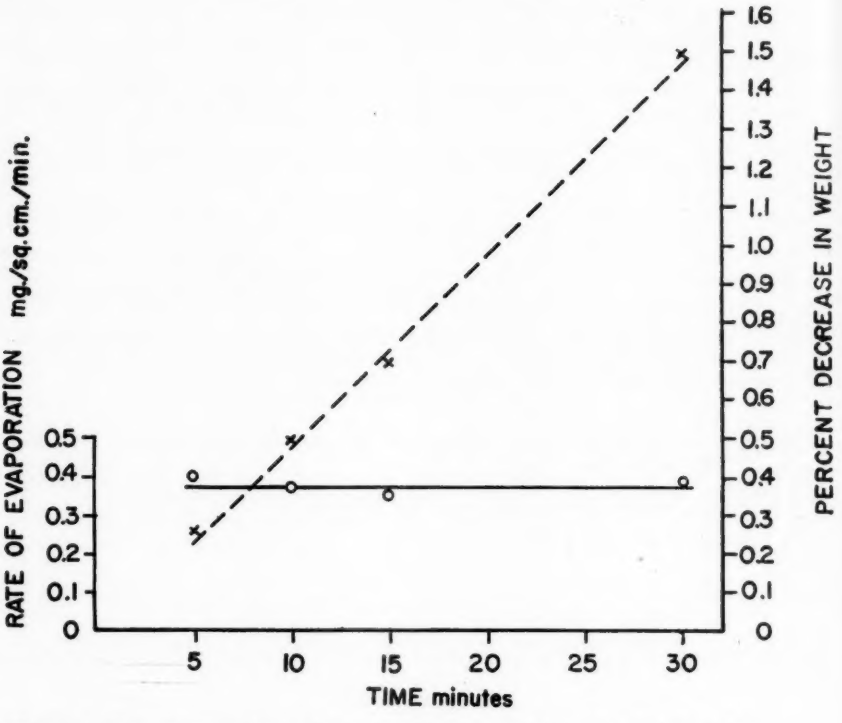

Figure 1. Rate of evaporation and percent decrease in weight as a function of time.

from each of five instars were used. All the experiments were performed on animals which had passed the third day after moulting. This was done to avoid complications which might arise from the interference of such processes as deposition and tanning of the cuticle.

The grasshoppers used in the first instar temperature series weighed 0.8-1.0 g. and had a total surface area of approximately 20 sq. cm. This total surface area of 20 sq. cm. was maintained as constant as possible throughout the course of these experiments by decreasing the quantity of grasshoppers used in each succeeding instar. Each curve presented in this communication represents the average of two independent trials.

The rate of evaporation per unit area was calculated from the amount of water lost by the insects and the area of the evaporation surface. The loss of body moisture was calculated from the difference of the weight of the insects prior to and after exposure to a dry atmosphere for a given period of time in an apparatus described elsewhere (4). The surface area was calculated according to Chefurka and Pepper (5). Loss of body moisture through the various body openings was prevented by sealing them with cellulose paint. Because the rate of moisture loss from freshly killed grasshoppers with spiracles sealed did not differ significantly from those whose spiracles were open, only the spiracles of the third, fourth, fifth instars, and the adults were sealed.

The evaporation from cuticular membranes and from a free water surface was determined using an aluminum cell of approximately half inch diameter and depth. The cell was fitted with a screw cap, the centre of which had a hole of the same diameter as the inside of the cell. The cap with its narrow flange fitted tightly against the brim of the cell. It was thus possible to place a membrane over the open end of the cell and have it held securely in position by the screw cap. The area of the evaporating surface was 0.916 sq. cm.

Results

1. *Rate of Evaporation from an intact cuticle.*

It was necessary to determine an exposure period that would allow for repeated determinations of moisture loss on the same batch of grasshoppers at different temperatures without reaching the point where all the free water was depleted. This optimum exposure period was ascertained by exposing a batch of grasshoppers to dry air for varying periods of time at 40°C.

From Figure 1 it will be seen that the rate of evaporation was constant for the various exposure times tested. Hence the equilibrium rate of vapor flow was apparently established very rapidly. Therefore, all subsequent determinations of water loss were performed by weighing the animals after exposing them for fifteen minutes to dry air. Furthermore the data plotted in Figure 1 show that the per cent decrease in weight increased linearly with time. This was taken as justification for the conversion of rates of evaporation to an hourly basis. Similar results were obtained when fresh lots of dead grasshoppers were exposed to dry air for periods of time varying from one-half to four hours.

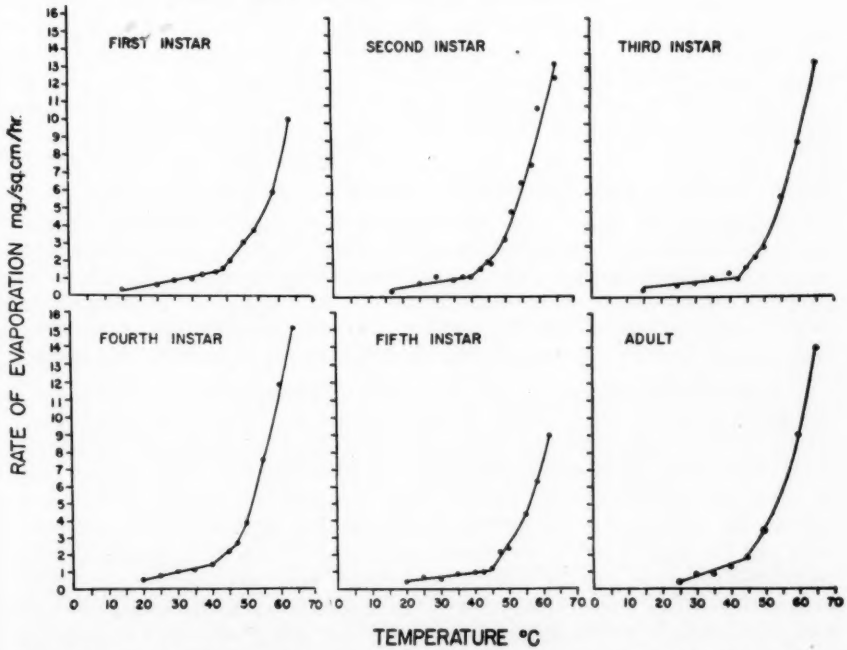

Figure 2. Rate of evaporation from grasshoppers in various instars as a function of temperature.

2. *Permeability of the cuticle of grasshoppers in various instars.*

The rates of evaporation-temperature plots, seen in Figure 2, indicate no significant difference in evaporation from dead grasshoppers in the various instars. The slight variation might be attributed to difference in the thickness and tanning of the cuticle of these insects both within each instar and between instars.

At ordinary temperatures, the rates of permeation through the cuticle were very low. Even at upper limits of this insect's temperature tolerance, the rate, under these experimental conditions, was approximately 1 mg./cm.^{2}/hr. Because this evaporation occurred under conditions of extremely low humidity, it seems evident that the amount of water which would be lost by this insect under field conditions would probably be negligible. This relatively high impermeability of the cuticle is due to the presence of a lipid layer in the outer surface of the epicuticle (4).

Most of the evaporation curves in Figure 2 have three regions of discontinuity. The evaporation rate rose suddenly at 43°C. and continued to increase linearly up to 50°C. Beyond this temperature the increase in the rate of evaporation was exponential. This linear increase in the rate of evaporation in the temperature interval 43°-50°C. is attributed to a structural change of the lipid epicuticle which will be the subject of the following communication. This temperature interval will be subsequently referred to as the "transition region" for the lipid epicuticle.

3. *Changes in the permeability of the cuticle after moulting.*

The data presented in Figure 3 show that the cuticle of a newly moulted grasshopper is more permeable that that of an older one. Thus, the rate of evaporation at 25°C. from a newly moulted cuticle was approximately two and a half times that from a 4-5 day old cuticle. These curves also show, that the greatest increase in cuticular impermeability occurred within 48 hours after moulting; the rate of evaporation being decreased by approximately 60% at 35°C.

4. *Permeability of chloroform extracted cuticles.*

Because of the rapid loss of water from grasshoppers treated with a chloroform-ether solution, it was impossible to perform the entire series of temperature-moisture loss determinations on the same lot of insects. Hence, one batch was used for determinations of moisture loss at 5°, 10°, and 15°C; another for 20° and 25°; another for 30° and 35°; and one for each determination at the highest temperatures. In all these trials the exposure time to dry air was twenty minutes.

A plot of these data as rate of evaporation *vs* temperature in Figure 4, shows the absence of any sharp break in the evaporation curves from grasshoppers whose cuticles were extracted with chloroform. A comparison of the rates of evaporation from grasshoppers treated in this manner with untreated first instar grasshoppers indicates that at 25°C., the rate of permeation through the untreated cuticle was 1/30 of that through the treated cuticle. It is also noteworthy that although the wax, above its transition region, permitted a free passage of water through the cuticle, yet the rate of evaporation never approached that from a cuticle which had been extracted with chloroform. Thus the presence of the wax, even though it was structurally disoriented, still conferred a considerable amount of water proofing on the cuticle.

5. Evaporation of water from a free water surface.

An examination of the rate of evaporation from a free water surface (Figure 4) shows that it is an exponential function of temperature. A comparison of the rate of evaporation from a free water surface and from a chloroform-treated cuticle shows that the difference in the rate of evaporation between the two was quantitative but not qualitative. Thus both curves were alike in all respects except for their relative vertical displacement. But when these curves are compared with the evaporation curve from an untreated cuticle, it is obvious that the evaporation from the latter is both qualitatively and quantitatively different from the former. The qualitative difference results from the occurrence of the transition region from 43 to 50°C. while the quantitative difference resides in the greatly reduced permeability of the untreated cuticle. Both these differences are due to the presence of the lipid epicuticle in the untreated cuticle.

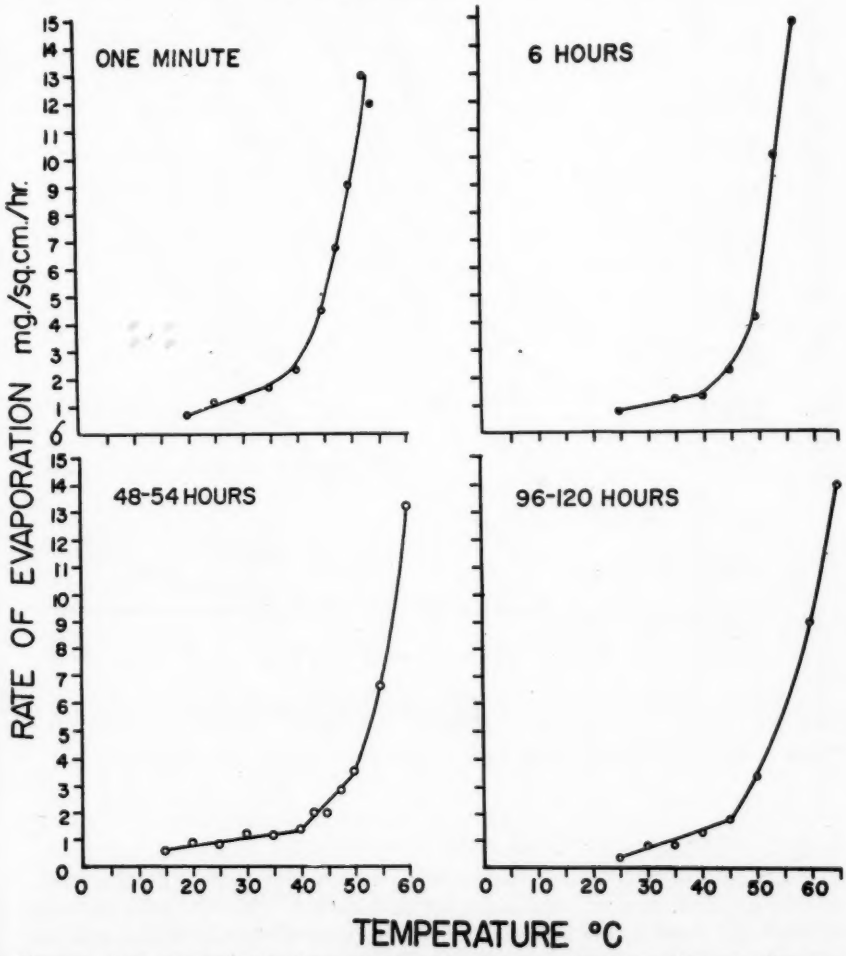

Figure 3. Rate of evaporation from adult grasshoppers at various times after moulting as a function of temperature.

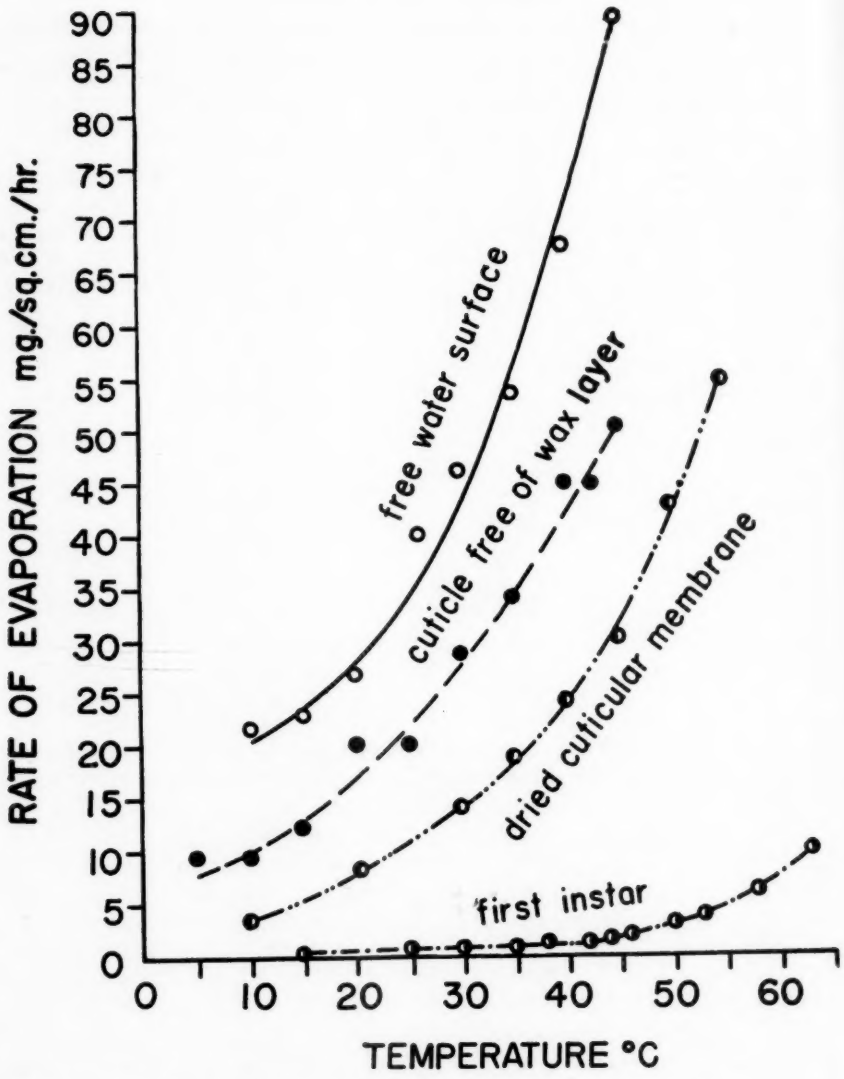

Figure 4. Rate of evaporation from various systems as a function of temperature.

6. Evaporation of water through cuticular membranes.

The rate of evaporation from a fresh cuticle and from a dried cuticular membrane, both free of wax, is shown in Figure 4. The rate of evaporation through the dried cuticular membrane was suppressed to a greater extent than through a similarly treated fresh cuticle. Presumably the drying process caused some change in the ultrastructure of the membrane rendering it less permeable.

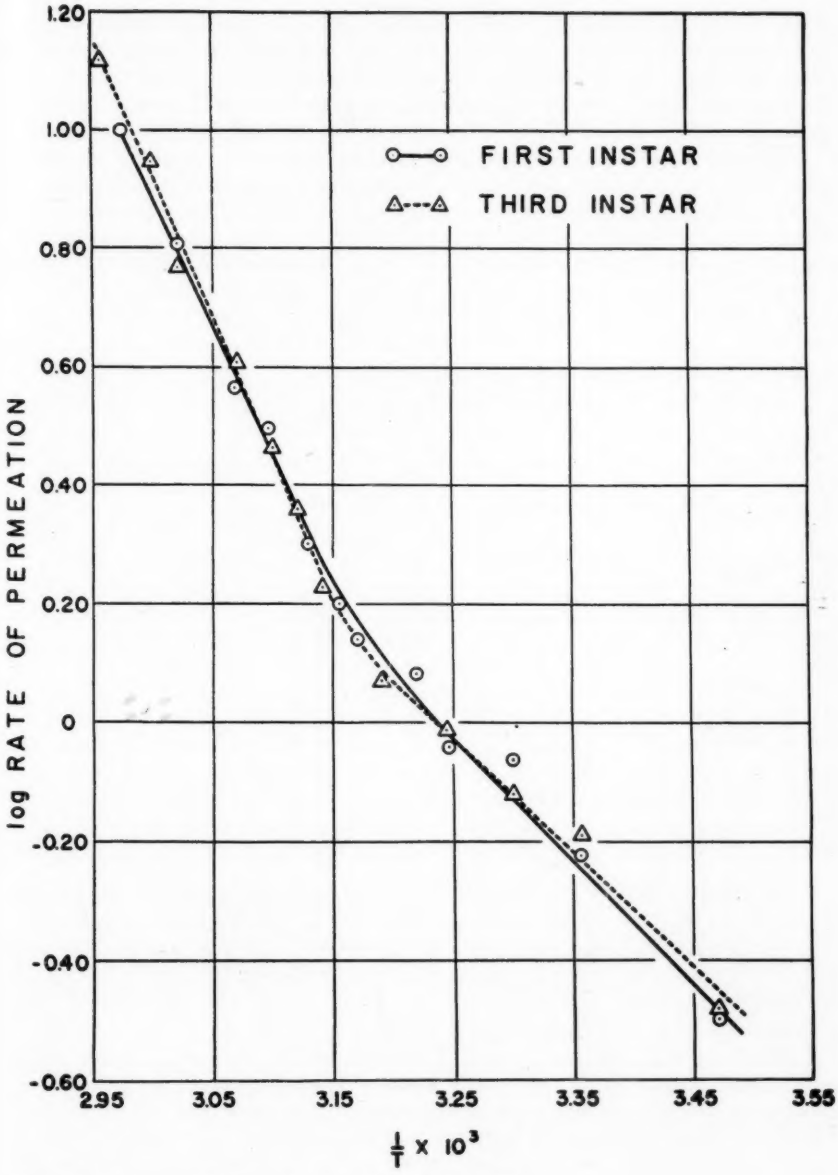

Figure 5. Log rate of permeation as a function of 1/T.

7. *Energy of Activation for Permeation.*

The energy of activation for permeation was calculated from the slope of the lines resulting from a plot of log rate of permeation *vs* $^1/_T$. A typical plot for two instars is seen in Figure 5. The straight lines are joined not by a break at a

single temperature but by a curve extending over a temperature interval of approximately seven degrees. It should be noted that good Arrhenius plots were obtained below the transition region of the lipid epicuticle. This suggests that the apparent linear plots in this region of Figure 2 should be exponential and probably result from insufficient sensitivity of our method of measurement.

The data for the activation energy for permeation for cuticles in various instars and in the adult stage at successive periods after moulting is presented in Table I. It shows that the energy of activation for permeation above the transition region is strikingly greater than below this temperature interval. Furthermore the energy of activation varies from 7.77 x 10^3 calories to 10.17 x 10^3 calories below the transition region. Above this region the variation is from 22.36 to 27.51 x 10^3 calories. The significance of this variation is at present not known.

TABLE I

Activation energies for permeation for cuticles in various instars and in the adult stage at successive periods after moulting. Expressed in calories x 10^3/mole

Stage of Development	Activation Energy for Permeation	
	Above the Transition Region (53-63°C.)	Below the Transition Region (25-40°C.)
First Instar	22.36	9.54
Second "	24.63	10.17
Third "	24.57	9.34
Fourth "	27.10	8.55
Fifth "	27.51	7.77
Adult		
one minute	29.20	12.00
six hours	28.60	8.04
48-54 hours	21.23	8.16
90-120 hours	21.88	11.95
Undried cuticular membrane free of wax	8.31	
Dried " " " " "	11.25	

The data in Table I also show that the energy of activation for permeation, above the transition region, decreases with ageing of the adult grasshopper. The greatest rate of decrease occurred within 48 hours after moulting. This may suggest that other physiological processes such as cuticular tanning and secretion are contributing to the permeability of the cuticle during this time and that these processes are essentially finished by 48 hours after moulting.

When the wax completely removed from the cuticle, the energy of activation for permeation was 8.31 x 10^3 calories whereas when a cuticular membrane was allowed to dry, the permeation of water through this membrane was suppressed and the energy of activation for permeation increased to 11.15 x 10^3 calories. It must be remembered that these data are not comparable with those for the untreated cuticle because the wax was still present on the untreated cuticle both above and below the transition region.

Discussion

(a) *Rate of evaporation from the cuticle.*

The results presented here show that the lipid epicuticle of the grasshopper *Melanoplus bivittatus*, as in other insects, is the main barrier to evaporation. If the

lipid epicuticle is removed by organic solvents such as chloroform, the cuticular permeability increases approximately 30 fold over that of the intact cuticle. It is true that the rate of evaporation from a chloroform-treated cuticle is still less than that from a free water surface but this suppression in evaporation is negligible when compared with that brought about by the presence of the lipid epicuticle.

There was no striking difference between the rate of evaporation from grasshoppers in the various instars. This coupled with the occurrence of the transition region of the wax at approximately the same temperature interval, suggests that the chemical and physical constitution of the lipid epicuticle did not change from instar to instar.

(b) *Transition Region of the Lipid Epicuticle.*

It has been shown by some investigators that the permeability of the lipid epicuticle increases at the so-called critical temperature (2, 17, 24). Beament (2) considers the rate of evaporation to increase at the transition point (apparently synonymous with critical temperature). The findings presented herein as well as those to be presented in the following paper for beeswax, indicate that complex insect waxes possess a broad transition region. The transition point is merely the lower temperature limit of the transition region.

The experimental evidence obtained for beeswax films to be reported in the following paper of this series, indicates that the transition region is the result of a gradual structural reorientation of the lipid epicuticle. High temperatures cause a gradual weakening of the forces which normally maintain the wax molecules in a quasi-stationary state. The molecules are then capable of increased oscillation until at sufficiently high temperatures they perform complete rotations about their main axes. Simultaneously with this increase in freedom of rotation, the molecular lattice probably assumes a different symmetry (15) and dimensions (16) providing for increased intermolecular spaces, which results in easier penetration of the lipid epicuticle by the water molecules.

It is noteworthy that Beament (2) noted optical changes and variations in the contact angle for insect waxes at temperatures several degrees below their melting points. He adopted the concept of molecular rotation suggested by Müller (15) to explain his findings. Slifer (20) noticed that the wax extracted from the eggs of *Melanoplus differentialis,* showed optical changes in the temperature interval 46-56°C which was well below its melting point of 60-65°C. The temperature limits of the transition region for wax of *M. differentialis* eggs were only a few degrees above that for the cuticular wax of *M. bivittatus*. Richards *et al* (17a) have shown that, after exposing larvae to temperatures ranging from 40-90°C, the rate of evaporation from *Sarcophaga* at 25°C increased only after exposing to temperatures 60-70°C which is presumably the transition region of the lipid epicuticle. Thus the temperature limits of the transition region are probably dependent on the chemical constitution of the wax. Furthermore this region is probably the α-form of the component fatty acids and esters of the lipid epicuticle but this point requires further investigation.

Investigations of the effects of temperature on the rate of biological and biochemical processes (6, 18, 19) as well as on the permeability characteristics of the cuticle (2, 17, 24) suggest that Arrhenius plots of such processes show sharp breaks at critical or transition temperatures. Kistiakowsky and Lumry (13) have challenged the occurrence of "breaks" in such plots by raising at least two serious objections. Firstly, because of severe technical difficulties, rate measurements

are, at best, accurate only to establish that two straight segments of a line are joined by a curved segment over a range of temperatures. Secondly, if the curvature were limited to one or two degrees the activation energies would have to differ by several hundred thousand calories. Our studies appear to lend support to these objections. In no instance was the difference in activation energy of sufficient order of magnitude to warrant the occurrence of a sharp break. Indeed, the straight lines were joined by a curved segment corresponding to the transition region of the wax. Thus the experimental evidence for the occurrence of a transition region for the lipid epicuticle in a rate of permeation–temperature plot for insects, as well as the theoretical considerations suggest that the current notion of "critical" temperature with respect to cuticular permeability is of dubious value. Hence, if a comparison of the rates of evaporation from various species needs to be made, it should be related to the transition region, or to its lower temperature limit, the transition point, which is a function of the properties of the wax, and not to any temperature at which the permeability reaches some arbitrarily assigned value such as 5 mg/sq.cm/hr. (24).

(c) *Activation energy for Cuticular Permeation.*

The data in Table I indicate that the energies of activation for permeation are about two and one half times as great above as below the transition region of the lipid epicuticle. If these data represent the energy required by the water molecules to penetrate the lipid epicuticle, then the implication is that the cuticle offered more resistance to water penetration above than below the transition region. Clearly, this is in contradiction to the experimental results which indicate that the cuticle is more permeable above the transition region of the wax layer. Indeed, these data for a complex biological system are a striking contrast to synthetic membranes (3, 7, 8, 9). Arrhenius plots for synthetic membranes show only one straight line. For natural rubber (8) Arrhenius plots show two straight lines but the magnitude of the energy of activation for permeation, as derived from the slope of the lines above and below the region of discontinuity, is the reverse of that presented here for a biological membrane. Nevertheless the occurrence of two lines of different slope joined by a curved segment suggests that the cuticle, as the natural rubber film, is essentially in different physical states with different properties above and below the transition region.

The shift from a low value of the energy of activation in the low temperature range to a high value in the high temperature range implies that the phenomenon of permeability is a composite one made up of several concurrent reactions which are differently influenced by temperature (11). The reaction rate in the lower temperature region with the smaller value for the energy of activation suggests the predominance of a heterogenous process; while in the higher temperature range with a higher energy of activation, a homogeneous process appears to be rate-limiting (11, 12, 22).

Although no precise definition of the participating reactions can be given at the present time, the existence of several possible processes has been recently brought to light by Doty (9). He found that the energy of activation for permeation of synthetic membranes could be resolved into the energy of activation for diffusion and heat of solution. Furthermore the energy of activation for permeation was the algebraic sum of the energy of activation for diffusion and the heat of solution. When the membranes were plasticized with tricesyl phosphate or fatty acids, the energy of activation for permeation changed. This change was mainly the result of a change in the heat of solution.

From these studies it appears that very little if any insight into the permeability properties of complex biological membranes can be gained by a mere consideration of gross permeability alone. Indeed Doty *et al* (8) reported that a polystyrene film had identical values for the energy of activation for diffusion and heat solution but opposite in sign giving an energy of activation for permeation of zero. Only when cuticular permeability is explored in terms of diffusion and solution and particularly the temperature dependence of these and other factors, will we get a better understanding of the fundamental nature of water vapor diffusion through complex biological membranes.

(d) *Rate of water as a function of age.*

In a previous paper of this series (4) it has been shown that the lipid epicuticle was secreted before the old exuvium was shed. This presumably is a protective measure against desiccation. The present data indicate further that the cuticle is most permeable immediately after ecdysis, and as the animal ages the cuticle becomes less permeable. This is in agreement with other studies (21, 23). According to the present data, the maximum impermeability was attained within 48 hours after moulting.

On the basis of present knowledge, it is difficult to ascertain the cause of the increasing impermeability after moulting. If the lipid epicuticle, which is intact during this period, was partly responsible for this decrease in permeability after ecdysis, then it must be due to some chemical or physical change in the wax. The wax exposed to the air after ecdysis may undergo polymerization or hardening, thereby becoming more impermeable. It may also undergo a reorientation towards closer packing of the molecules. If reorientation of the wax does occur after moulting, then it must be confined to the sub-surface molecules; otherwise, such changes should be reflected in the values of the contact angle of distilled water on the cuticle. It has already been shown that the contact angles remained essentially constant before and after shedding of the exuvium (4). It has been suggested (24) that increased impermeability may be brought about by the secretion of more wax, but it is difficult to see how this can be so, particularly if the findings of Beament, (2) and Alexander *et al*, (1), that the most effective thickness at the lipid-substrate interface, are accepted.

Changes in the physical and chemical state of the lipid are not the only factors which might be responsible for the increase in cuticular impermeability following moulting. During this period active secretion and tanning of the predominantly protein portion of the cuticle is in progress. Eder (10) and Lafon (14) have shown that both secretion of more cuticle and tanning can play a significant role in cuticular permeability. But what ever these processes may be, they probably play a relatively minor waterproofing role under the normal temperature conditions of the habitat of the insect. The lipid epicuticle appears to be the major factor in passive water regulation.

Summary

A study has been made on the effects of temperature on the rate of evaporation from the cuticle of *Melanoplus bivittatus*. No significant differences were noted in the rate of evaporation from cuticles in various instars. The rate of evaporation at 25°C. was very low. This is due to the presence of a discrete layer of wax on the cuticle. If this layer of wax is removed by organic solvents, the permeability of the cuticle increased thirty-fold at 25°C.

The cuticle becomes increasingly impermeable with time after ecdysis. The greatest decrease in the permeability of a cuticle occurred within 48 hours after moulting.

The evaporation curves showed three distinct regions. The so-called "critical" temperature is a transition point which signals the appearance of a transition region from 43 to 50°C. It is suggested that the increase in the rate of evaporation in the transition regions is due to disorganization of the lipid epicuticle. One feature of this disorganization is molecular rotation.

Energies of activation for permeation have been determined. There is a shift from a low value of the energy of activation for permeation in the low temperature range to a high value of the high temperature range. This implies that permeability of complex biological membranes is made up of two or more concurrent reactions which are differently influenced by temperature. It is suggested that these reactions may be diffusion and solution and any thorough treatment of membrane permeability must take these into account.

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III. On the Physical Nature of the Transition Region of Insect Waxes[1]

Introduction

It has now been well established that the cuticle of many insects is covered by a discrete layer of lipoidal material. Ramsay (26), Wigglesworth (32), Beament (4), and others have shown that the rate of evaporation-temperature curves from insects are characterized by breaks occuring at a "critical" temperature. In the previous paper of this series, Chefurka and Pepper (8) have shown that the rate of evaporation-temperature curves from *Melanoplus bivittatus* show a transition region from approximately 43 to 50°C.

Müller (21) noted that n-paraffins show crystalline changes at temperatures a few degrees below their melting points. Later he showed that at such temperatures the molecules are capable of rotation around the main axes and that along with molecular rotation, there occurs an increase in the volume of the crystal lattice (22, 23). Both Beament (4), and Chefurka and Pepper (8) have adopted this explanation for the increase in permeability of the lipid epicuticle at the transition point and in the transition region respectively.

In this paper, we wish to present experimental evidence for our view. Because a sufficient quantity of the cuticular wax from *M. bivittatus* was not available, beeswax was used as experimental material. It has been shown recently (28) that cuticular lipids from exuviae of silkworms have a similar composition to beeswax.

Methods and Materials

(a) Measurement of the Dielectric Constant.

A General Radio model 716-C Schering type, capacitance bridge was employed for the measurements. The bridge was energized by a Hewlett-Packard oscillator which was continously variable from 30 cycles to 300 kilocycles. Bridge

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balance was obtained by the use of a balancing-capacitor, Precision Condenser, type 722D, along with an amplifier and null detector type 1231-A.

The beeswax samples were measured in a condensor cell of the Smyth type*. It had a capacitance of 436.1 uuf at 25°C. and a capacity of 100 ml. It consisted of three concentric gold-plated brass cylinders held rigidly in position by small ceramic spacers. The inner and outer cylinders remained at high potential. The outer cylinder was the containing vessel. The cell was covered by a brass lid held in place by several screws. The temperature of the cell contents was recorded with a thermometer inserted through a hole in the lid of the cell, the bulb of the thermometer reaching to the centre of the cell. Water at any desired temperature was circulated through the outer cylinder from a constant temperature bath. All measurements were made at a frequency of one kilocycle.

Beeswax samples were liquified and poured into the cell. Air bubbles were eliminated by stirring during hardening and by subsequent reheating and resolidification. The temperature of the wax was then varied and when equilibrium was reached the necessary measurements were made. The data presented here are those accrued during heating of the wax. The values for the cell constant, the capacitance of the cell filled with benzene and for the capacitance of the empty cell at the various temperatures were obtained from calibration curves. The variation of the dielectric constant of benzene, freshly distilled over sodium, was also obtained from a previously determined calibration curve.

The cell constant was calculated by means of the following formula:

$$C = \frac{C_B - C_E}{E_B - E_A}$$

where C = cell constant

C_B = capacitance of the cell out of the circuit-capacitance of the cell filled with benzene

C_E = capacitance of cell out of the circuit-capacitance of empty cell

E_B = dielectric constant of benzene

E_A = dielectric constant of air = 1

Once the cell constant was calculated, the dielectric constant of beeswax was obtained by the formula

$$E_{BW} = \frac{C_{BW} - C_E}{C} + E_A$$

where C_{BW} = capacitance of cell out of the circuit-capacitance of cell filled with beeswax.

(b) Relationship between film thickness and permeability.

The thickness of besswax which would give maximum suppression of evaporation was determined by depositing a known amount of beeswax solution in chloroform from calibrated syringe onto a cuticular membrane freed of its lipid epicuticle by washing in chloroform.

This membrane was placed in the aluminum cell previously described (8). Assuming that the wax distributed itself evenly over the membrane surface, the thickness of wax was calculated by knowing the concentration, the amount deposited, the area of the surface covered and the density of the wax which was assumed to be 0.96 gm/cc (18).

The results presented in Figure 1, indicate that a thickness of film greater than two microns had little effect in further retarding the evaporation. The rate

*As modified by Dr. C. N. Caughlan of the Chemistry Department.

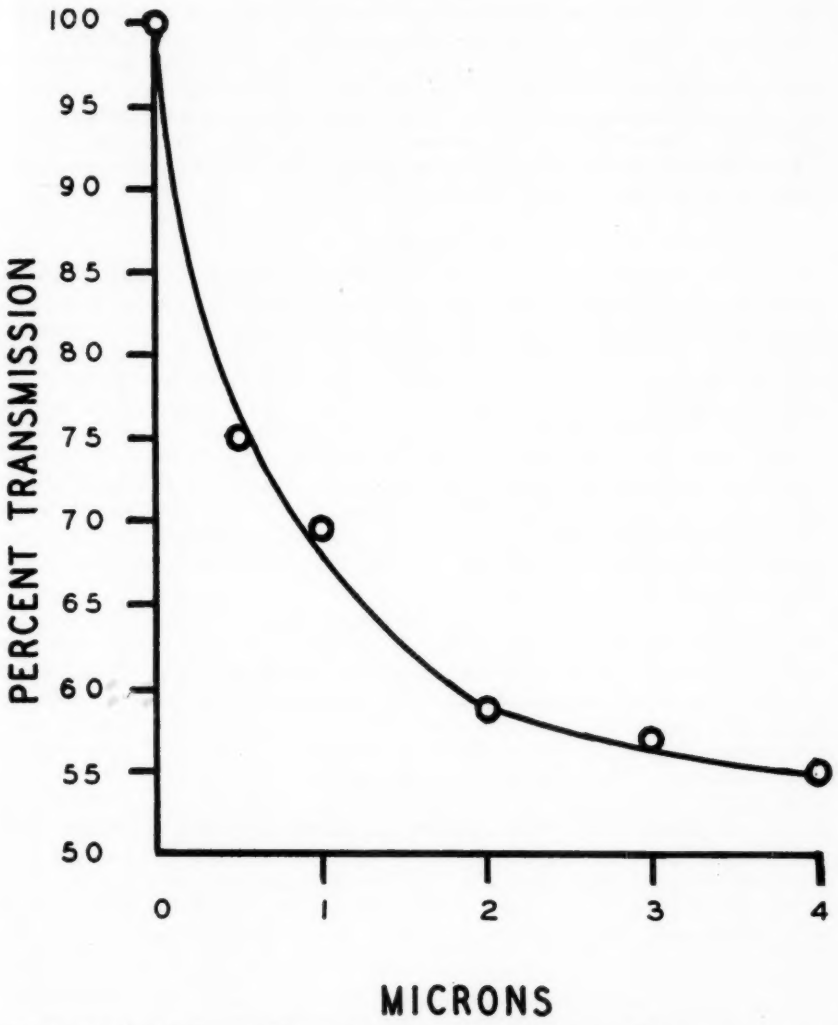

Fig. 1 Relationship Between Evaporation and Thickness of Beeswax Film.

of evaporation was decreased by approximately fifty per cent by a film thickness of 0.5 microns which corresponds to approximately 50 monolayers (33). This confirms the findings of Alexander *et al* (1) and Beament (4). Presumably it is the lowermost oriented layers of wax that are most effective in retarding evaporation.

(c) Measurement of the Permeability of the Film.

The permeability measurements at various temperatures were made using the aluminum cell and apparatus previously described (8). The membranes were portions of the abdominal cuticle of *Melanoplus bivittatus* prepared as described

previously (8). The lipid epicuticle was removed by extraction in cold chloroform for several minutes and a two-micron-film of beeswax was deposited on the membrane as outlined above. The cell, filled with distilled water and with the membrane held securely between the rim and the screw cap, was allowed to equilibrate at room temperature for several hours before any measurements were made. The distilled water in the cell was never in contact with the inner side of the membrane; a saturated air space separated the water from the membrane. All determinations were performed in duplicate.

Results

In 1930, thermal evidence led Pauling (24) to suggest that molecules with low moments of inertia are able to rotate when the crystal approaches its melting point. He predicted that when such molecules are polar, the dielectric constant of a crystal at the high temperature should behave like that of a pure liquid. In other words, increased freedom of rotation would be made manifest by dielectric constants equal to or higher than the refraction value (square of the optical refractive index) of the liquid. Presumably molecular rotation arises from a gradual weakening of the intermolecular forces permitting the molecules greater freedom of rotation. Since then it has been demonstrated by numerous investigators that this view is applicable to such solids as long-chair paraffins (2), esters (3, 7, 11), bromides (15, 16), alcohols (3, 17), solid solutions of ketones and paraffins (13), symmetrical ketones (22, 23), and non-aromatic compounds and certain benzene derivatives (30, 31).

If molecular rotation does occur in beeswax then its dielectric constant in the solid state should be similar to that of the liquid and both should be larger than the square of the index of refraction. The data presented in Table I show that the dielectric constant of the solid was similar to that of the liquid and was significantly larger than the refraction value of the liquid. This indicates that some molecular rotation or at least oscillation occurs in the solid state even at room temperature. Furthermore, the polarization of the solid at 25°C is at least as high as that of the liquid and 75.5 higher than the molar refraction of the liquid. This is a further indication that a not inconsiderable freedom of molecular rotation or oscillation exists in the solid even at room temperature. The fact that this difference between molar refraction and polarization is even greater at 51.6°C, and indeed reaches its peak value at this temperature (Figure 2), indicates that at this temperature most of the molecules are in a rotational state.

TABLE I

The dielectric constant, refraction value, molar refraction and polarization at several representative temperatures taken from figure 2.

Temperature °C.	ϵ	n^{2*}	M.R.[1]	M.P.[1]
25.2	2.762	-		266.0
39.3	2.711			258.1
51.6	2.835			273.2
64.5	2.675	2.08–2.09	190.5	264.8

* Handbook of Chemistry and Physics 35th ed. 1953–54.

1. Assuming that the major contribution to rotation was by mericyl palmitate M.W. 691.2. Average density of beeswax 0.96 (18).

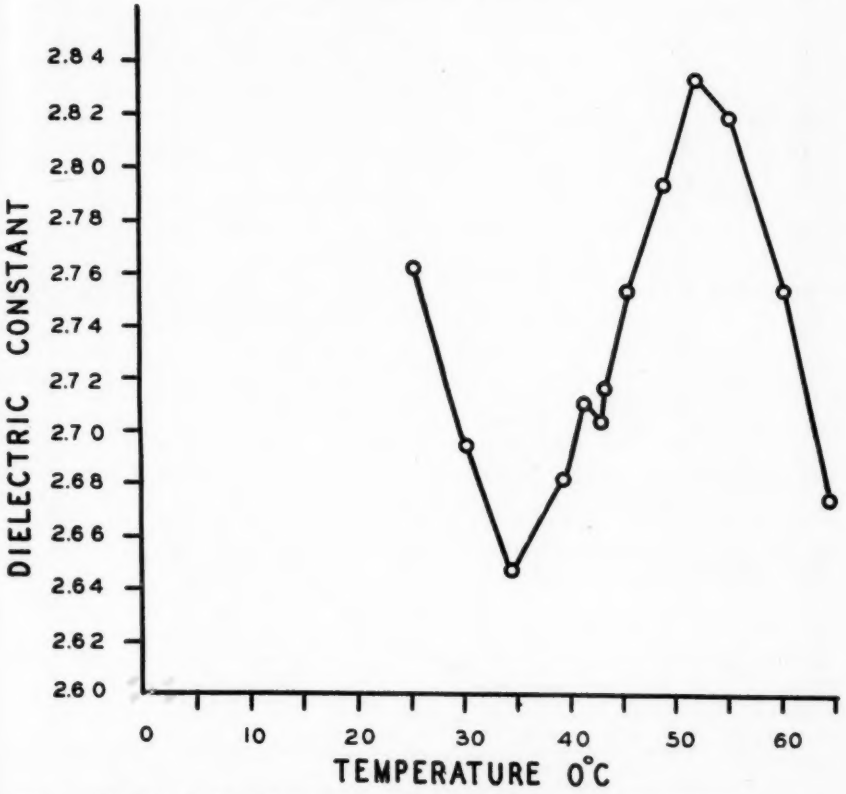

Fig. 2. Variation of the dielectric constant of beeswax at various temperatures.

These findings are in agreement with those of Crowe *et al* (12) on long-chain esters. If we assume, in view of the relatively sharp melting point of this sample of beeswax (64-65°C), that the main component is mericyl palmitate then the symmetry value of this molecule as defined by Crowe *et al* is 17. These investigators found evidence for molecular rotation in all esters of similar symmetry.

The data in Figure 2 show that with increasing temperature the dielectric constant drops. This decrease with rising temperature may suggest that, because of increased freedom of motion, the increase in thermal agitation more than compensates for the increase in freedom of molecular rotation. At 35°C, the dielectric constant rose sharply indicating a change in structure from one with relatively immobile to one with mobile dipoles. For pure compounds this change in structure is usually completed within several degrees. For a substance of the complexity of beeswax however, the change in dielectric constant occurred gradually over a temperature interval, which is probably a composite of the transition points of the individual components of beeswax.

It is well known that fatty acids and their esters pass through at least two crystalline changes during a wide temperature change (19, 25). On cooling from the liquid state the α-form separates out as a transparent colorless solid. On

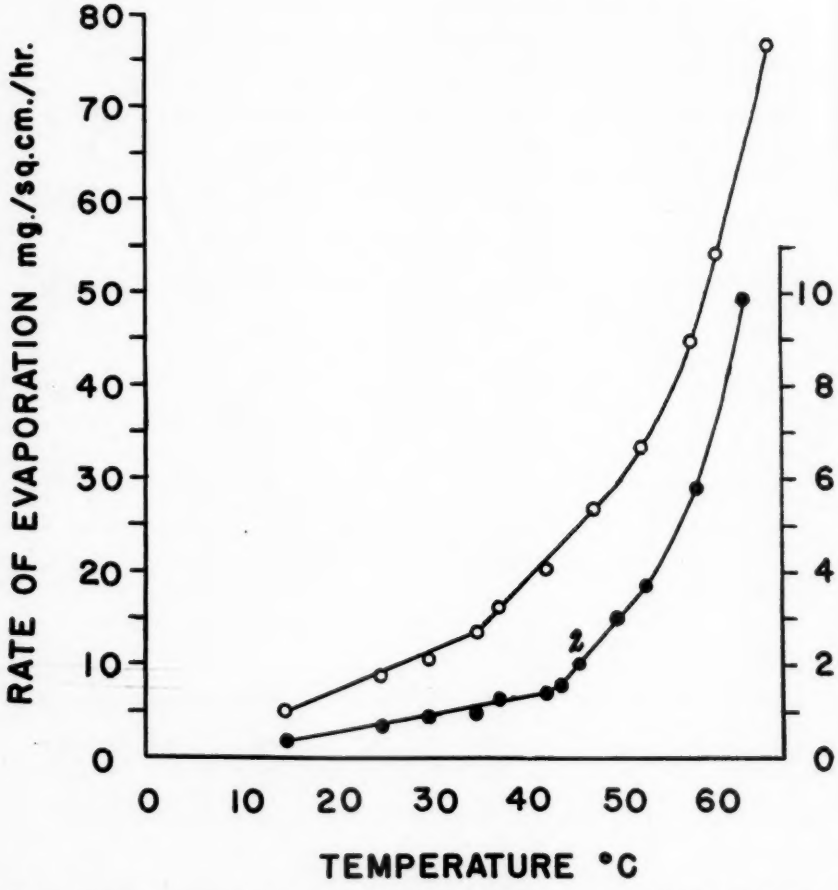

Fig. 3. Rate of evaporation as a function of temperature. Curve 1 (left hand scale)-cuticular membrane covered by a film of beeswax. Curve 2 (right hand scale)—first instar grasshopper.

standing or further cooling the α-form passes into the β-form. X-ray studies reveal that the molecules in the α-form possess chains usually in the vertical position while the chains in the β-form are usually tilted (19), Woog and Yannaquis (33) have shown that beeswax undergoes a change in lattice dimensions in the temperature range 29-38°C. The a, b, and c axes increased in size but the greatest increase occurred in the length of the long or c axis. Since Motz and Trillat (20) have shown that the molecular chains of beeswax are tilted at room temperature the findings of Woog and Yannaquis probably indicate a gradual vertical orientation of these chains (19a). Our findings localize this transformation point from the β-state to the α-state at 34.5°C. Furthermore, the occurrence of molecular rotation in the α-state is in agreement with the body of evidence which indicates that molecular rotation occurs only when the chains are in the vertical position (19a).

(b) Evaporation from Beeswax Films.

The data in Figure 3 (curve 1) show that the rate of evaporation from beeswax films increased suddenly at 35°C. and continued to increase linearly to 51°C., beyond which the increase was exponential. No discontinuity in the evaporation curve was noted at the melting point. Hence the increase in evaporation at the transition point of beeswax occurs approximately 30° below the melting point and coincides with onset of extensive molecular rotation.

Qualitatively the evaporation curve from beeswax is similar to that from a first instar grasshopper cuticle covered by the lipid epicuticle (Figure 3, curve 2). Both show the presence of the transition region. The temperature limits of the transition region are different however, probably because of the difference in chemical composition of the waxes.

Discussion

The data presented in this paper reveal that the increase in evaporation in the transition region of beeswax films is correlated with molecular rotation. As the temperature increased, the forces between adjacent wax molecules were weakened allowing the molecules to oscillate. At high temperatures the forces were sufficiently weakened to permit a complete rotation. Simultaneously with the increase in molecular rotation, the lattice dimensions of the wax molecules increased (23, 33), allowing for increased intermolecular spaces. Thus the total energy of the potential barrier was decreased and the water molecules were therefore able to penetrate the film more readily.

Insect waxes are known to be composed predominately of acids, esters, alcohols and paraffins (5, 6, 9, 10, 14, 27). It has been shown (Figure 3, curve 2) that cuticular waxes pass through a transition region similar to that of beeswax. Furthermore, Beament (4) noted optical changes and variations on contact angles for insect waxes at temperatures several degrees below their melting point. Slifer (29) noted that the wax from the cuticular shell of eggs of *M. differentialis* showed distinct optical changes in the region 46-56°C. The melting point of this wax was 60-65°C. Recently Richards *et al* (26a) have reported that the rate of evaporation from *Sarcophaga* at 25°C was not affected by exposure, for relatively short times, to temperatures from 40-60°C and from 70-90°C. However, within the temperature range 60-70°C, the rate of evaporation increased linearly. Our findings provide an explanation for this phenomenon. In the temperature interval 40-60°C, the wax molecules are in a state of oscillation, the extent of which is dependent upon the temperature. However, the degree of oscillation is not sufficient to overcome the intermolecular forces and hence, when the temperature is returned to 25°C, the wax molecules assume practically the same orientation as they had prior to exposure. However, in the temperature range 60-70°C, the wax molecules pass through the transition region by rotation about their axis and probably by a change in orientation from the β- to the α-state. Hence each time the temperature is returned to 25°C the molecules become "fixed" in a new state of orientation and close packing with the result that the permeability is different from what it was at temperatures below the transition region. Having attained the α-state, any subsequent exposure to higher temperatures eg. 70-88°, has no further effect on molecular rotation and orientation and hence the rate of evaporation again does not change. The sudden change in the rate of evaporation which they find at 88°-92°C may be due to a change in phase from the solid to the liquid or to some change in the structure of other cuticular components.

In summary therefore, we suggest that the occurrence of various phenomena in the transition region of insect waxes is the result of molecular rotation and lattice changes similar to that shown here in the transition region of beeswax. Thus the magnitude of the rate of evaporation in the transition region as well as the changes in certain other properties of insect waxes in their transition region, is dependent in part upon the kinetic state of the wax molecules. Whether or not this transition region is synonymous with the α-form of the lattices of the components of insect waxes must be decided by future investigations.

Summary

The rate of evaporation-temperature curves from cuticular membranes covered by a film of beeswax show a transition region similar to that from intact cuticles. It has also been shown by measuring the changes in the dielectric constant with temperature, that in the transition region of beeswax the wax molecules rotate around their main axes.

The correlation between the increase in evaporation and molecular rotation in the transition region indicates that the phenomenon of increased permeability of the insect cuticle in the transition region is due to a disorganization of the tightly packed wax molecules of the epicuticle as a result of thermal weakening of the intermolecular forces. In this state of molecular orientation with its expanded lattice size, the water molecules penetrate the lipid epicuticle more easily.

Acknowledgments

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Towards An Insect Ecology[1]

By JOHN A. CHAPMAN[2]

It cannot be disputed that in any attempt to control populations of useful or harmful insects a knowledge of their ecology is of prime importance. In view of the significance of the various relationships between man and insects it follows that insect ecology should be a vigorous and strongly supported phase of entomology. It is the purpose of this discussion to consider briefly the present status of insect ecology and its relationship to ecology in general, and particularly to call attention to those characteristics of insect life that would seem to justify special treatment of insects from an ecological standpoint.

A statement that insect ecology is not a relatively well developed phase of entomology may be questioned. One can point to two books which deal primarily with this aspect of ecology (Chapman, 1931; Folsom and Wardle, 1934), to the fairly widespread inclusion of insect ecology courses in institutions offering training in entomology, and to the considerable extent to which basic and applied entomological problems involve ecological methods and considerations. Also, it is true that many general ecological studies include some treatment of insects.

A judgment on the present status of this branch of entomology must be a relative one and will depend on personal viewpoints and definitions, and because ecology is a field of study which depends on the factual bases supplied by the other subdivisions of biology insect ecology must lag, to some extent, behind other phases of entomology. Nevertheless, to the writer it appears that insect ecology is in a relatively weak position and that this weakness cannot be accounted for by its nature alone. While every entomologist knows something about ecology there are too few insect ecologists, if we include in this category those working with insects who devote their major efforts to securing or applying the basic facts and principles of ecology.

It is, of course, to be hoped that the recent rapid advances on the insecticide front can be continued, and it must be gratifying to all entomologists interested in ecology that there is strong current interest in insect physiology (see Chauvin, 1949; Wigglesworth, 1950; Roeder et al., 1953). One cannot help wishing, however, that insect ecology showed comparable indications of vigour at present.*

The results of our continued heavy dependence on chemical control in dealing with harmful insects is viewed by some with apprehension (e.g., Pickett, 1949). Is it sound to allow insect ecology to lag as an approach to insect problems? Should not the systematic study and analysis of the basic aspects of the ecology of insects and the acquisition of further knowledge in this field be placed on at least as strong a footing as the study of their physiological mechanisms or the special consideration of the action of various poisons on them? Does the marked discrepancy between the support of chemical control studies and the support of ecological studies speak well for the future outcome of man's competition with the insects?

One may grant the importance of ecology in approaching insect problems and still raise the question, why an *insect* ecology? Such a question is logical

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*Since this paper was written in final form the extensive treatment of ecology by Andrewartha and Birch (1954) has appeared. Their work is largely based on insect studies and is indeed a fresh and vigorous approach to insect ecology.

since ecology represents an integrating approach to the study of life, and all life is interrelated with other forms of life, as well as with the non-living environmental factors. It is true that animal ecology has been dealt with as a whole for some time (Allee *et al.*, 1949). However, there is justification for considering insect ecology apart from animal ecology.

First there is the need for an analytical approach, even to ecology. The working units of an ecological study are distinct entities of the living world and in practice must, of necessity, be considered in terms of some taxonomic specialization. The numbers and economic importance of the Insecta long ago led to the development of entomology as a branch of zoology. For the same reason any ecological approach to insect problems can profit by existence of a specialized phase of ecology, namely insect ecology. Furthermore, insects are interrelated with their environment in many ways characteristic of them and sometimes peculiar to them. It is true that other arthropods are similar to insects in various ways. However, as far as man is concerned the insects are easily the most important and must be considered far more frequently.

Before presenting and discussing those features of insect life which are of particular ecological significance it should be stressed that we are medium-sized warm-blooded terrestrial vertebrates and that of necessity we always begin by viewing the world as it affects our own bodies, as it is revealed by our own senses, and as it is interpreted by our own brains. This gives us an unescapable bias in considering insects. We must continually remind ourselves that these organisms represent a very successful but very different pattern of life than our own, and that some environmental factors may influence them quite differently than they influence us.

The points mentioned in the following section are grouped under various headings although it is realized that the various parts of the pattern of insect life are so interrelated that any separation of them, for purposes of discussion, is an artificial one.

a. *The extent and variety of genetic patterns in the insects.*

At many taxonomic levels there is structural evidence of a large reservoir of genetic potential in the insects. As a general rule, orders, families, and genera include many categories relative to other groups of organisms, and this comparison may be extended to the specific and subspecific levels. This structural variety is undoubtedly paralleled or exceeded by functional variations. Insects have utilized available terrestrial and fresh water habitats as completely as any group of organisms. They show, as might be expected on the basis of their numbers and variety, a tremendous number of special interrelationships among themselves, both interspecific and intraspecific, and with viruses, microorganisms, plants, and other animals (e.g., Sweetman, 1936; Clausen, 1940; Steinhaus, 1946; Buchner, 1953). The great variety of plant and animal material which is utilized as insect food is likewise evidence of the extent of structural and functional variability to be found among the insects (Brues, 1946).

The recent events on the chemical control front, where marked inheritable resistance against many insecticides has appeared in various insect populations (Knipling, 1953, for example) has given clear indication of an aspect of insect life which must never be lightly considered, namely adaptability at the species level. Such adaptability is dependent on potential variability within the large pool of genetic material available in most insect species and also on the possibilities of change in this material due to mutation.

b. *High degree of cellular specialization in insects.*

The insect, like the vertebrate, stands at the pinnacle of an evolutionary peak. This status of the insect group reveals itself at the cellular and tissue levels in the large number of structural and functional specializations which show considerable complexity. For example, the insect integument with its associated structures is a remarkably efficient and specialized structure based upon a complex biochemical system (Richards, 1951). Again, highly developed sensory systems and very efficient motor mechanisms of various types are found in insects, and the group shares with other arthropods and with vertebrates the possession of hormone regulation of various body processes, a fairly advanced condition considered from an evolutionary standpoint. Structural and functional adaptations to particular environmental situations are frequently found among the insects. Considered as living mechanisms, the insects are intricate, highly specialized, and very efficient, and well suited to exist under many of the conditions which prevail on the earth's surface.

c. *Biotic potential.*

The potential of insects to increase under ideal circumstances is great and is generally far above that encountered in the terrestrial vertebrates. This has many implications which must be carefully considered in dealing with insects. High biotic potential probably is one of the bases of their genetic adaptability. For example, it provides the means through which favourable gene combinations can be quickly reproduced. It provides an important basis for their population variations, that are frequently of great magnitude and are often of concern to us. It is an important factor in relegating chemical control to the category of a temporary measure, in most cases. Much importance must be attached to attempts to understand and evaluate this high potential and to determine the natural factors which usually influence it.

d. *Body size of insects.*

The relatively small body size of insects as compared with many other animal groups, and particularly with the vertebrates, is such a commonplace part of human experience that even entomologists probably seldom consciously consider its many implications. Body size affects the food requirements of an organism in many ways in terms of amounts and types of food which can be utilized effectively. Size also appears to be related to length of life cycle and thus to biotic potential. In addition, it is a factor which influences physiological and physical processes in a variety of ways (Thompson, 1942). For example, the respiratory system of insects is of a type that functions most efficiently for organisms below a certain range of body size (Krogh, 1941). Internal distribution of food, oxygen, and excretory products is much less a problem to small organisms than to larger forms due to size-volume relationships. An exoskeleton can more efficiently perform the function of structural support in small organisms than in larger forms. The problem of the periodic shedding of the integument, with its attendant immoblity, is probably less serious to small organisms than it would be to large forms. Size-volume-surface relationships also influence other aspects of the life and activity such as relative muscular strength, heat exchange with the environment either by conduction or radiation, water exchange with the environment, and flight and involuntary aerial distribution. Temperature exchanges with the environment are of particular importance because insects are poikilothermic animals. Because of size alone, insects can be said to live in a different environment than man.

e. *Effective habitat of insects.*

Man is hardly a creature of the microclimate, as considered by Geiger (1950). Although technically he lives within this zone of the atmosphere most of his sensory organs are located near its upper limit. Also, he frequently so modifies his environment as to be isolated from the direct effects of climate much of the time and this is bound to influence his own awareness of the importance of climatic factors and their variations. On the other hand, the insect is typically an inhabitant of the microclimate or the plant climate. The justification for considering these regions as distinct from the climate lies primarily in their frequently considerable differences from it in moisture, temperature, and air currents. As pointed out above, the influences and relative importance of these factors are related in many ways to the size range of the organism concerned.

Micromicroclimates, such as those found on a rock surface, on or within a leaf, in the cambium region of trees, or in many other very localized areas inhabited or frequented by insects, likewise present characteristic combinations of meteorological conditions which may differ markedly at times from the climate. Thus the meteorological factors which directly affect insects may be considerably different from those of which man is immediately aware and which are usually measured by him. The differences may exist for both extreme values and integrated values of the physical factors involved. Yet it is the immediate or effective habitat of the insect which must be properly measured and evaluated in any ecological studies of these organisms. The problems of techniques and instruments for measuring elements of the insect habitat are special and very important ones to the insect ecologist.

f. *Wings.*

Only four groups of organisms have at some time during their evolution developed the power of true flight; the reptiles, the birds, the mammals, and the insects. Of all the tremendous number and variety of invertebrates, insects alone can fly. The presence of wings may profoundly influence success in meeting each of those very basic and important requirements of food, self-protection and reproduction. Moreover, possession of wings permits considerable flexibility in selecting the most suitable of the available physical environmental conditions. Ability to fly greatly facilitates species distribution, particularly in view of the size range of insects and the importance, relative to this, of the influence of air currents. The view that wings evolved early and but once during the history of this group is well established, and it would appear that these structures were an important factor in the tremendous development of this group of organisms. There are, of course, other common features of the insect pattern of life, such as the division of main body regions and the presence of three pairs of legs, but at least it is certain that wings were a part of that ancestral pattern which has given rise to the vast majority of present-day forms and it is highly probable that presence of wings has contributed much to the biological success of insects.

g. *The life history from an ecological standpoint.*

Some kind of metamorphosis is to be found in most insects and all of the four largest orders have a life history involving four separate stages, two of which do not feed and, with the exception of certain pupae, do not move about. The ecological significance of the egg and pupal stages may be considerable as, in addition to being periods of structural change and reorganization, both may serve as resistant stages bridging the time gap between periods of unfavourable

environmental conditions. For example, times of food shortage or of certain moisture or temperature conditions unsuitable for the active forms may thus be tolerated by a species.

In insects with complete metamorphosis the larvae and adults frequently have almost completely different ecological requirements. Such multiple environmental needs by one species might be expected to have disadvantages and certainly to provide some limitations to species distribution. Nevertheless, judging from the biological success of those insects having active stages which live in different ways and in different habitats, such an arrangement must often actually contribute to that success. One way in which such a life history might be advantageous is that it permits specialization as to function within the life cycle as well as within the individual. For example, growth can be accomplished in one body form, specialized for efficient and rapid food consumption, while mating and species distribution can be carried out by another, and structurally different, stage.

Metamorphosis complicates the study of the ecological requirements of an insect species. Because of the difference in form, habitat, and environmental requirements of the various stages ecological studies on an insect may present far more difficulties than such a study of a vertebrate. However, it is of much importance in approaching insect problems to have fairly complete ecological information on single species.

h. *Role of instinctive behaviour in insect activity.*

In dealing with insects we can take some consolation from the fact that, although species adaptability seems to be a general feature of insect life, certainly individual adaptability is not. The learning process is probably of relatively little importance in the life of insects, as compared with its role in the higher vertebrates. The extent and arrangement of neural capacity and the time available for learning, somewhat related to the body size and length and nature of life cycle respectively, are probably both correlated with the development of a considerable dependence on fixed inherent, or instinctive, responses in insects. Instinctive behaviour in these forms may be relatively simple or quite complex. However, variability of individual responses is not so apt to be attributable to the past experience of the individual involved as in the mammals, for example, with their far better ability to learn, i.e., to modify their behaviour on the basis of previous experience. Since the organism is far from an inert part of the interrelationships between it and the environment, this characteristic of insect life is of much ecological importance.

In this discussion attention has been given to insect ecology as a field of knowledge and endeavour. Those structural and functional characteristics of insects which are important in any ecological consideration of them are presented and briefly discussed. It is believed that these characteristics should be well understood and constantly considered by the insect ecologist because they form major parts of the pattern of insect interrelationships with the environment. It is felt that there is need for greater interest in, and support of, insect ecology by entomologists and by agencies responsible for meeting insect problems.

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Certain Host-Parasite Relationships Involving the Spruce Budworm[1]

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Introduction

During studies of the parasite complex of the spruce budworm in the Lake Nipigon region of northern Ontario from 1946 to 1950, data were obtained on certain host-parasite relationships. The data were derived from two sources: the rearing each year of periodic collections of spruce budworms, and the dissection of budworm larvae and pupae that were collected and preserved at 2-day intervals during June of 1948 and 1949. The particular features investigated were: the sex ratio of parasites and its possible relation to the sex of the host; and the effect of parasitism on the apparent sex, rate of development, and size of the host.

Methods

The rearing program was developed primarily to yield information on the incidence and relative abundance of various species of parasites as natural control agents. However, the details are of little concern here, since only a part of the data illustrate particular features of host-parasite relationships. Suffice it to say that samples of spruce budworm larvae and pupae were collected from a series of plots at four specific periods of host development each year. At the time of collection, the insects were subdivided by apparent sex and instar into groups which were kept intact throughout the subsequent rearing. This allowed the end results of the rearing to be related back to the sex and stage of the budworms at the time of collection.

The dissection data were obtained from samples of approximately 100 larvae or pupae taken at one of the sample points at 2-day intervals during June and July of 1948 and 1949. The material was killed and fixed in Peterson's KAAD mixture and later stored in 95 per cent alcohol. As each budworm was dissected, the sex was determined, the head-capsule width measured, and the presence or absence of parasites noted. A total of 2,268 larvae and 396 pupae were dissected.

Most larvae were easily sexed by observing the gonads. Female gonads remain undeveloped throughout the larval and early pupal periods whereas the male gonads begin to develop in the early larval stages and appear as two deeply pigmented bodies embedded in the fat body dorsally in the fourth or fifth abdominal segment. The sex of small larvae and of some parasitized individuals could not be satisfactorily established by this means. Recourse was made to a cytological method (7, 8) which takes advantage of the fact that female Lepidoptera are heterogametic and their somatic cells show the sex chromosome, even during the resting stage, when stained with aceto-carmine. The large nuclei of the salivary glands are particularly adaptable to this test. A simple staining procedure allowed an examination of these nuclei for the presence or absence of the heavily stained inclusion which identified females. Technical difficulties or the lack of suitable tissue prevented sex determination in a few instances.

Head-capsule widths were determined under a compound microscope using an ocular micrometer. The measurement taken was the maximum width to the nearest one-fiftieth of a millimetre.

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TABLE I

Sex Ratios and Associated Data for Three Larval Parasites of the Spruce Budworm, 1947–1950

Parasite	Year	No. of parasites:		Sex of parasites			Sex of hosts from which emerged:			Stages of host from which emerged:				
		Obtained	Per cent emerged	♂	♀	Ratio	♂	♀	?	III	IV	V	VI	?
Apanteles	1947	152	69	34	71	1:2.1	–	–	–	–	–	–	–	152
	1948	14	78	1	10	1:10	–	–	–	–	8	3	–	3
	1949	57	70	19	21	1:1.1	–	–	–	7	25	24	–	1
	1950	155	61	42	52	1:1.2	–	–	–	36	90	25	–	4
		378	66	96	154	1:1.6	–	–	–	43	123	52	–	160
Glypta	1947	410	72	155	139	1:0.9	–	–	–	–	–	–	–	410
	1948	105	69	22	51	1:2.3	–	–	–	–	–	31	74	–
	1949	140	77	52	56	1:1.1	–	–	–	–	–	27	102	11
	1950	187	74	52	87	1:1.7	–	–	–	–	–	94	85	8
		842	73	281	333	1:1.2	–	–	–	–	–	152	261	429
Meteorus	1947	205	64	57	75	1:1.3	–	–	–	–	–	–	–	205
	1948	296	84	107	143	1:1.3	106	190	–	–	–	–	296	–
	1949	435	74	134	187	1:1.4	196	236	3	–	–	5	430	–
	1950	242	84	83	121	1:1.5	83	156	3	–	–	3	239	–
		1178	78	381	526	1:1.4	385	582	6	–	–	8	965	205

The identification of the immature stages of the parasites was attended with certain difficulties. Descriptions of the immature stages of *Apanteles fumiferanae* Vier. (2) and *Glypta fumiferanae* (Vier.) (3) aided in their identification. Another distinct type which appeared regularly was considered to be *Meteorus trachynotus* Vier. The remaining parasites were simply classed as dipterous or hymenopterous.

Results

Parasite Sex Ratios

The sex ratios for the more common hymenopterous parasites obtained in the rearing program appear in Tables I and II. Table I shows the data for the larval parasites, *Apanteles*, *Glypta*, and *Meteorus* and includes information on their hosts. The sex of budworms parasitized by *Apanteles* and *Glypta* is omitted because of complications discussed below. The sex ratio of *Apanteles* and *Glypta* varied somewhat from year to year, while that for *Meteorus* was reasonably consistent. In all cases, the ratios indicated a slight predominance of females.

Information on the stage of the host from which the parasites were recovered also appears in Table I. The visual estimation of the instar of living larvae is difficult and particularly susceptible to error when larvae are parasitized. The groupings in the table, therefore, must be interpreted as being more indicative of host size than instar. It is apparent, however, that *Apanteles* larvae issue primarily from fourth-and fifth-instar budworm larvae as shown in previous more critical work (6); *Glypta* larvae issue from host larvae apparently in the fifth and sixth instar; and *Meteorus* almost exclusively from sixth-instar host larvae.

Table II presents the sex ratios and associated data for three pupal parasites, *Apechthis ontario* (Cress.), *Itoplectis conquisitor* (Say), and *Phaeogenes hariolus*

(Cress.). It has been reported (4) that the sex of hymenopterous parasites is influenced by the sex of the host from which they emerge. This has been considered a response to host size and results in female parasites coming primarily from female hosts and male parasites from male hosts. The present data were examined with this in mind by applying a chi-square test to the distribution of male and female parasites among male and female hosts. The first test concerned the relative occurrence of the four possibilities; male parasites from both male and female hosts, and female parasites from both male and female hosts. When a significant difference was found, as with *Apechthis* and *Phaeogenes*, a second more critical test was applied to the occurrence of males from males and females from females against males from females and females from males. *Apechthis* showed a significant difference ($\chi^2 = 4.78$ at 1 d.f.) and *Phaeogenes* a highly significant difference ($\chi^2 = 14.6$ at 1 d.f.). Apparently the sexes of *Apechthis* and *Phaeogenes* do not occur at random and are associated with the sex of the host. The most frequent combinations were male parasites from male hosts and female parasites from female hosts. This relationship was most critical in the case of female hosts since male parasites were rarely recovered from female hosts. Better agreement would perhaps be obtained if the weights of the pupae were known, for the weight range of female pupae, although generally larger, overlaps that for males.

It should also be noted that all three pupal parasites showed a consistent decline in the relative abundance of females from 1947 to 1950 which coincides with the general decline of the host population.

Influence of Parasitism on the Apparent Sex of the Host

The use of the presence or absence of visible male gonads to separate living budworm larvae into male and female groups soon resulted in an apparent anomoly. Almost every *Apanteles* and *Glypta* parasite was recovered from a "female"

TABLE II

Sex Ratios and Associated Data for Three Pupal Parasites of the Spruce Budworm, 1947–1950

Parasite	Year	Number obtained	Sex of parasite: / Sex of host:	Male ♂	Male ♀	Male ?	Female ♂	Female ♀	Female ?	Sex ratio ♂'s to ♀'s
Apechthis	1947	18		–	–	4	–	–	14	1:3.5
	1948	23		7	3	–	5	8	–	1:1.3
	1949	49		17	3	–	15	14	–	1:1.4
	1950	14		7	3	–	2	2	–	1:0.4
		104		31	9	4	22	24	14	1:1.4
Itoplectis	1947	25		–	–	7	–	–	18	1:2.6
	1948	2		1	–	–	1	–	–	1:1.0
	1949	12		3	3	–	2	4	–	1:1.0
	1950	31		11	5	–	6	9	–	1:0.9
		70		15	8	7	9	13	18	1:1.3
Phaeogenes	1947	69		–	–	27	–	–	42	1:1.6
	1948	30		10	3	–	7	10	–	1:1.3
	1949	36		17	3	–	6	10	–	1:0.8
	1950	2		–	–	–	–	2	–	–
		137		27	6	27	13	22	42	1:1.3

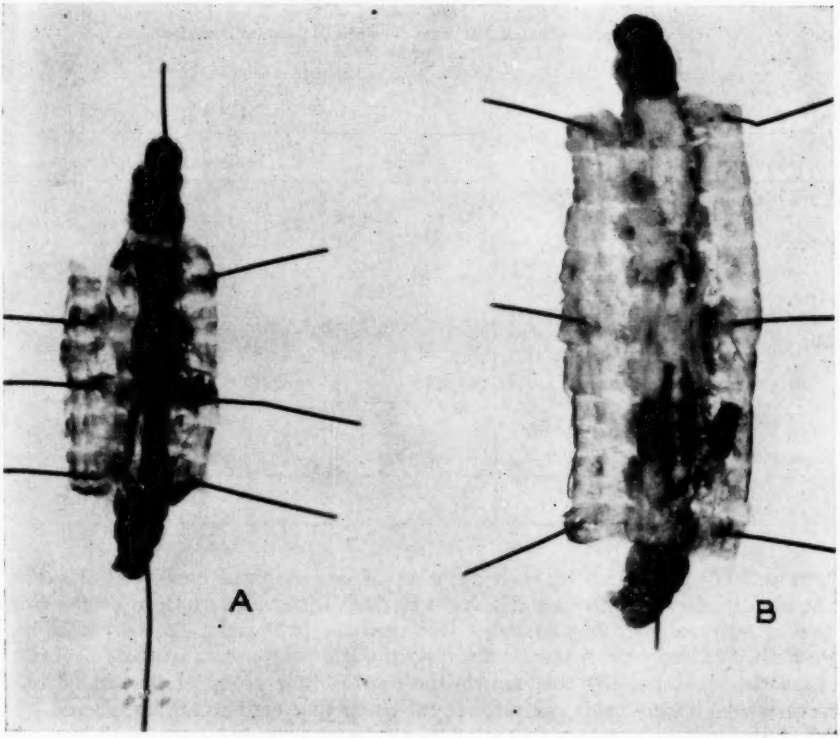

Fig. 1. Dissections of two male spruce budworm larvae collected at same time and place. Larva A contained a parasite, *Glypta fumiferanae* (Vier.); larva B was unparasitized. Note differences in size, particularly the gonads.

larva. The most tenable explanation was that these parasites, which become associated with the host early in its development (2, 3), directly or indirectly prevent the growth of the male gonads. During dissection, it was found that larvae containing these parasites were invariably small, contained little fat body, and the male gonads were poorly developed. These undeveloped gonads could not be discerned from the exterior and in consequence such larvae would be classed as females. The striking retardation in gonadal development is evident in Fig. 1 which shows two dissected male sixth-instar larvae which were collected from the same place on the same day. One larva contained a well-advanced *Glypta* larva; the other was not parasitized. The sexing of all dissected host larvae resulted in the distributions of parasites between the two sexes of the host shown in Table III. Obviously budworm larvae of both sexes were parasitized by *Apanteles* and *Glypta*.

Influence of Parasitism on the Rate of Host Development

A noticeable retardation of development by certain parasites became evident when host collections were separated into instar groups at the time of collection. Most *Apanteles* and *Glypta* were from host larvae in the least advanced instars at the time of collection. Rearing records indicated that the appearance of *Apan-*

TABLE III

The Distribution of Parasites Between Males and Females in 2,268 Dissected Spruce Budworm Larvae

Dissection results	Sex of host larvae:		
	Male	Female	Unknown
Parasitized by:			
Apanteles	113	102	5
Glypta	156	101	4
Meteorus	27	52	–
Diptera	10	14	–
Hymenoptera	16	19	12
Total parasitized	322	288	21
Unparasitized:	784	850	3
Totals:	1,106	1,138	24

teles and *Glypta* coincided with pupation of unparasitized hosts. Fig. 2 shows the incidence of *Apanteles* and *Glypta* in the various instar groups of the first two of series of four regular collections made in 1949 and 1950. Larvae in the least advanced stages contained a disproportionately large share of these parasites. *Apanteles* was especially abundant in the fourth-instar group of the second collections which were made just prior to the emergence of this parasite species.

Such effects are not restricted to *Apanteles* and *Glypta*. *Meteorus* influences the development of the host which it attacks during the late fifth or early sixth instar. Parasitized larvae stop feeding about the time unparasitized larvae are pupating. Shortly afterwards the parasite larva issues from the host without killing it and forms a cocoon within the host's feeding shelter. The budworm larva continues to live for as much as a week or more but does not feed or leave the shelter. One of the fourth collections made in 1949 provides an excellent example of the effect *Meteorus* has on host development. The collection was made when the first budworm adults were appearing and consisted of 165 sixth-instar larvae and 173 pupae; the larvae resulted in 111 *Meteorus* and 5 other parasites, while 33 died, and only 16 pupated. The collection was made late enough that some of the 33 larvae that died could have been parasitized by *Meteorus*. It is apparent, however, that the majority of larvae, still present when moths were beginning to emerge, were parasitized by *Meteorus*.

Influence of Parasitism on the Size of the Host

In studies designed to establish criteria for the separation of larval instars of the spruce budworm (6), it was shown that discrete instar distributions on the basis of head width do not result from the measurement of random samples of field-collected larvae. A better separation of instar groups was obtained when male and female larvae were segregated, and highly significant differences in mean head-capsule width were demonstrated between the sexes in the fifth and sixth instars. The hypothesis was established that the elimination of parasitized

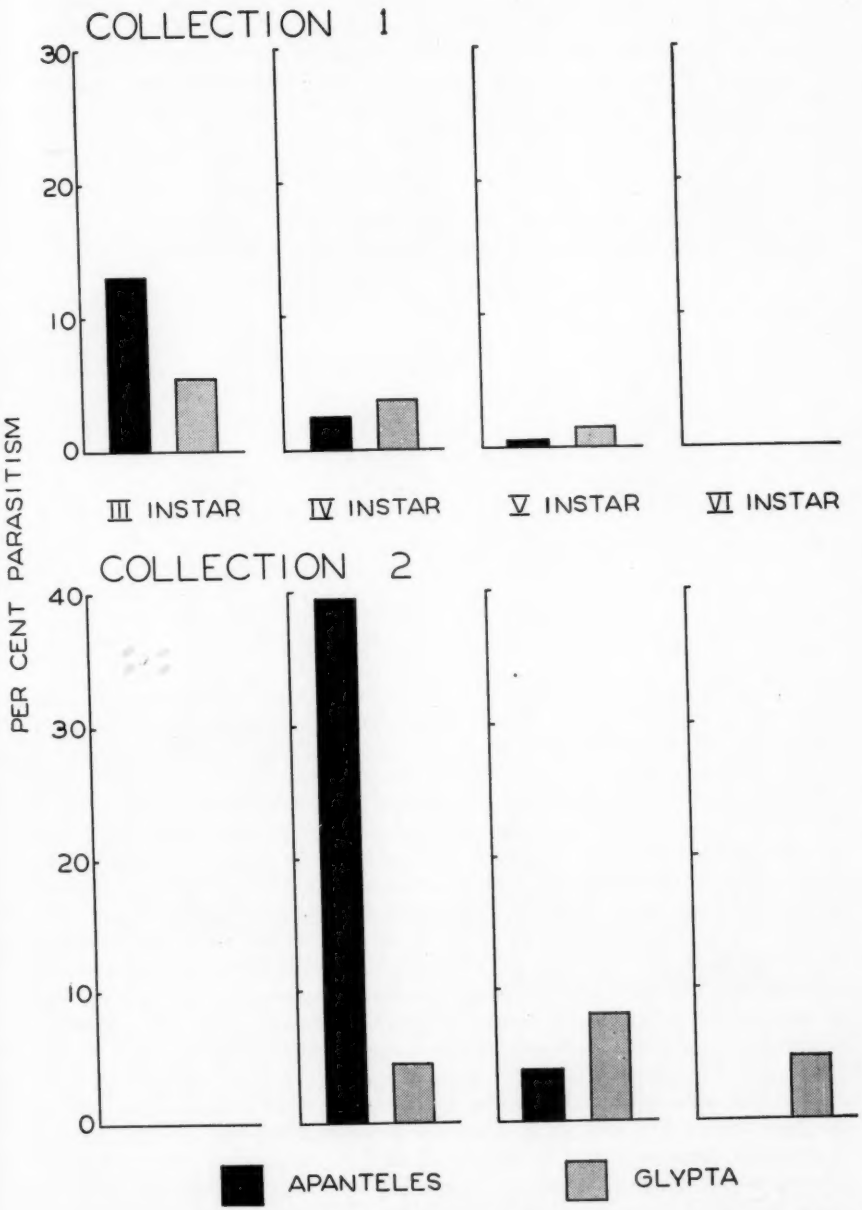

Fig. 2. Bar graphs showing the percentage parasitism by *Apanteles fumiferanae* Vier. and *Glypta fumiferanae* (Vier.) in the instar groups of all first and second collections made in 1949 and 1950. The instar groups are based on the stage at time of collection.

individuals from the samples would have resulted in a much better definition of instars on the basis of head width. Therefore, the head-capsule widths of all dissected larvae in the 1948 collections and the parasitized larvae in the 1949 collections were measured and the results examined for evidence to support this hypothesis. Measurements were made of 1,124 unparasitized budworm larvae and 552 larvae parasitized by *Apanteles*, *Glypta*, and *Meteorus*. The larvae ranged from the third to the sixth instar.

TABLE IV
Head-Capsule Size of Unparasitized Male and Female Spruce Budworm Larvae

Instar	Sex	Head width statistics:			
		No.	Mean (mm.)	Standard deviation	t
IV	♂	109	0.635	0.051	3.415**
	♀	122	0.660	0.060	
V	♂	149	1.039	0.067	11.63**
	♀	153	1.129	0.068	
VI	♂	257	1.662	0.082	20.26**
	♀	322	1.803	0.084	

The frequency distributions of head width are shown in Fig. 3. The distributions for male and female larvae are shown separately owing to the highly significant differences between the two sexes (Table IV). Except for the small group of female larvae between the fourth and fifth instar and of male larvae between the fifth and sixth instar, the unparasitized larvae show much more discrete instar groupings than were obtained previously (6). These small intermediate groups could have resulted from overlooking parasites in dissection. It was found that their inclusion with either instar did not influence the statistical results and they were, therefore, eliminated from the calculations. The distributions for parasitized larvae were not subdivided by sex since comparisons of means had indicated that there were no significant differences between the sexes.

The instar means for larvae parasitized by *Apanteles* and *Glypta* were compared with the corresponding instar of unparasitized male larvae. Any significant differences would also be applicable to unparasitized females because of their larger size. The results (Table V) indicate that larvae parasitized by *Apanteles* and *Glypta* have appreciably smaller head capsules than unparasitized larvae of the same instar. The differences are more pronounced in the later instars (e.g. fifth instars parasitized by *Apanteles* and sixth instars parasitized by *Glypta*). The instar separation of larvae that contained *Apanteles* was not arbitrary. Previous individual rearings (6) had shown that budworm larvae parasitized by *Apanteles* develop to the fourth instar before any parasites emerge and that some larvae do moult to the fifth instar with a negligible growth increment prior to parasite emergence.

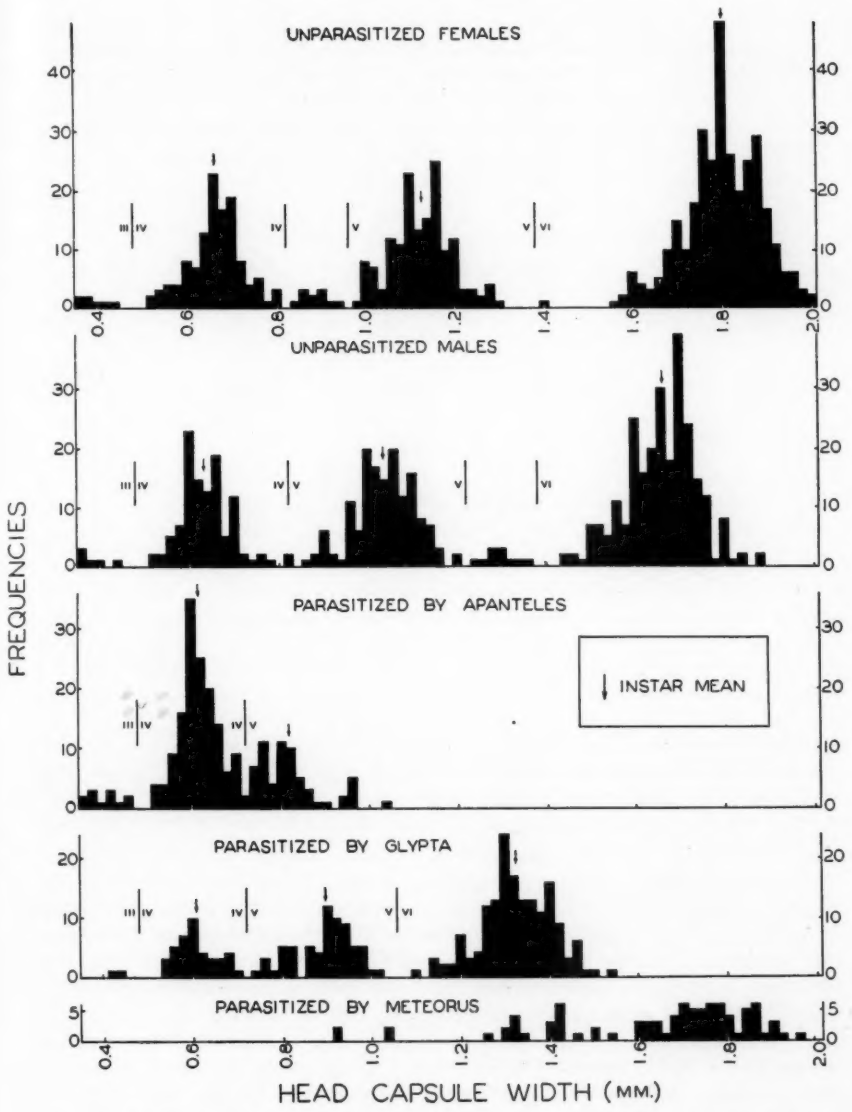

Fig. 3. Histograms of head-capsule widths of male and female unparasitized spruce budworm larvae and of budworm larvae parasitized by *Apanteles fumiferanae* Vier., *Glypta fumiferanae* (Vier.), and *Meteorus trachynotus* Vier.

It was not considered advisable to subject the head widths of larvae parasitized by *Meteorus* to statistical treatment because of the comparatively small sample and the tentative nature of the determinations of the immature stages of this parasite. Parasitism by *Meteorus* apparently has a much less pronounced effect on the head-capsule size of the host.

TABLE V

Head-Capsule Size of Parasitized and Unparasitized* Spruce Budworm Larvae

Instar	Head width statistics:			
	No.	Mean (mm.)	Standard deviation	t
IV (Unparasitized)	109	0.635	0.051	3.27**
(Apanteles)	142	0.616	0.042	
IV (Unparasitized)	109	0.635	0.051	3.12**
(Glypta)	40	0.607	0.043	
V (Unparasitized)	149	1.039	0.067	21.73**
(Apanteles)	63	0.816	0.070	
V (Unparasitized)	149	1.039	0.067	14.43**
(Glypta)	67	0.894	0.070	
VI (Unparasitized)	257	1.662	0.082	41.71**
(Glypta)	162	1.325	0.078	

*Only data for unparasitized male larvae used in statistical comparisons; see text.

Discussion and Conclusions

The data presented on the interrelationships of host and parasite sex tend to corroborate observations of similar relationships by other authors and, as suggested by Clausen (4), may be of considerable importance in the colonization of parasites for biological control purposes. The sexes of the pupal parasites, *Apechthis* and *Phaeogenes*, did not occur at random among male and female hosts as there was a definite tendency for female parasites to emerge from female hosts and male parasites from male hosts. This is possibly a response to host size as female budworm pupae are somewhat larger than males. The sex ratio of other hymenopterous parasites varied considerably from year to year with a slight decrease in the proportion of females as the infestation subsided.

It has been shown that the presence of the parasites, *Apanteles* and *Glypta*, has a marked effect on spruce budworm larvae. Their presence invalidates a sexing technique based on external evidence of male gonads, by inhibiting gonadal development. They materially retard the rate of larval development to the extent that *Apanteles* emerges from fourth- and fifth-instar larvae and *Glypta* from fifth- and sixth-instar larvae, when the bulk of the budworm population has reached the pupal stage. They influence the size of spruce budworm larvae so that the normal sex difference in head-capsule size is eliminated and the head-capsule size is highly significantly different from unparasitized larvae of the same instar. These influences have been presented separately but are interrelated manifestations of the influence of parasitism and its decided influence on the metabolism of the host. The relative intensity of the effects produced by the two parasites is correlated with their habits. The most decided effects are produced by *Apanteles* which is the first to mature and emerge from the host.

The small retarded larvae that result from parasitism by *Apanteles* or *Glypta* are easily overlooked in field samples, particularly when their incidence is low. Their omission seriously affects the appraisal of these species as natural control factors. The timing of aerial spray operations is usually based on the instar composition of periodic collections. Large numbers of small parasitized larvae could be a definite complication.

Meteorus and the various dipterous parasites do not produce such striking results as *Apanteles* and *Glypta*, although *Meteorus* does have an unusual effect on the host. The host larvae are not killed directly but are prevented from pupating. Such larvae are still present when unparasitized hosts reach the adult stage. In general, it appears that parasites which attack the host late in larval development do not influence head-capsule size or gonadal development to the same extent as parasites which attack the host in the early larval stages.

The effect of parasitism on head-capsule size is of interest in the light of findings by Beck (1). Working with the European corn borer and purified diets, he showed that Dyar's Rule of geometric growth ratios had no fundamental basis. Rather he established an allometric relationship between body weight and head-capsule size and concluded that the weight of a larva at the time of moulting controlled the growth increment rather than the absolute size of the head capsule. The significantly different trends in head-capsule size between parasitized and unparasitized larvae demonstrated above are similar to the results Beck produced with suboptimal diets.

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A Vibration-Resistant Instrument Stand[1]

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Occasions frequently arise in laboratory work when it is necessary to insulate a delicate piece of apparatus, such as a sensitive balance, from vibration. Various tables designed to eliminate vibration have been described (anonymous). Most of these tables require the attachment of iron supports to a load-bearing wall or the sinking of a support into bed rock. Occasionally the building construction or the type of underlying strata, or both, make such methods of mounting ineffective. The instrument stand shown in Fig. 1 requires no attachments. It consists of a

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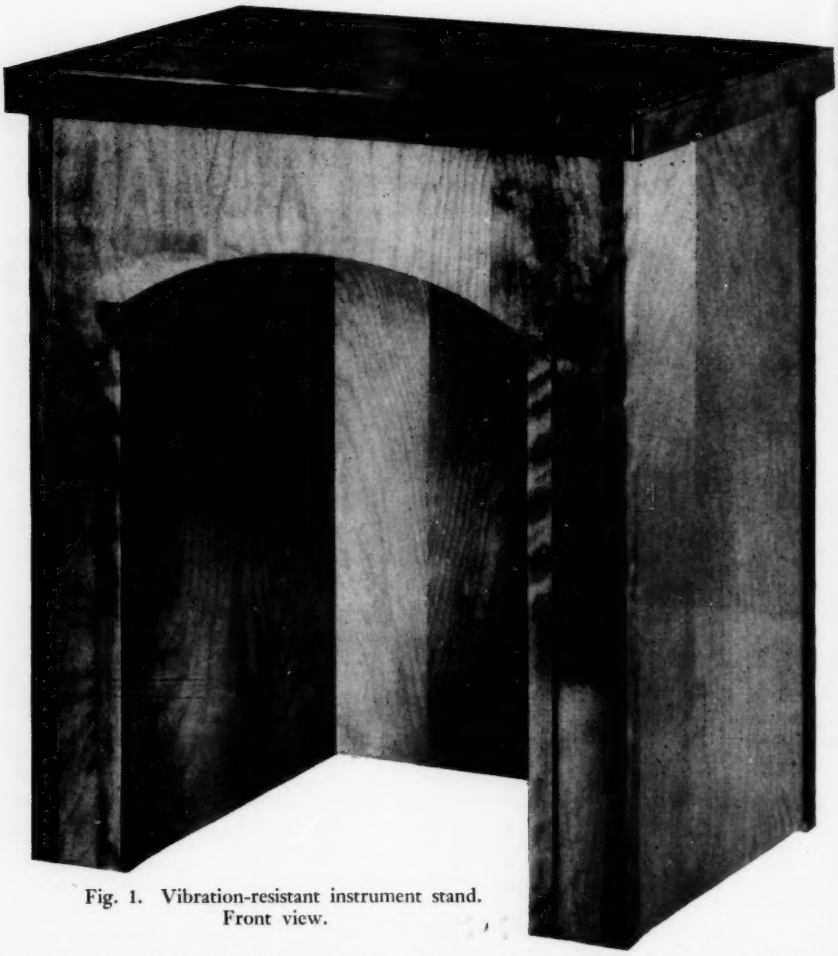

Fig. 1. Vibration-resistant instrument stand. Front view.

rigid table 34 inches high, 30 inches wide, and 18 inches deep. The legs are made from 4 x 4 inch lumber sheathed with quarter-inch plywood to form hollow sides; these sides are loaded with lead shot, steel stampings, or any other easily handled, heavy material. When loaded, the table weights can easily be removed through openings at the tops of the hollow sides, under the cover.

Vibration is reduced by setting two rubber stoppers, size 13, under the foot of each leg. No vibration could be detected in a semi-micro balance sensitive to 0.2 milligrams when it was set on this instrument stand even though the floor vibration was very severe.

Acknowledgments

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Reference

Anonymous. Planning a balance table to lick vibration. *Laboratory* 23 (1): 31. 1953.

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